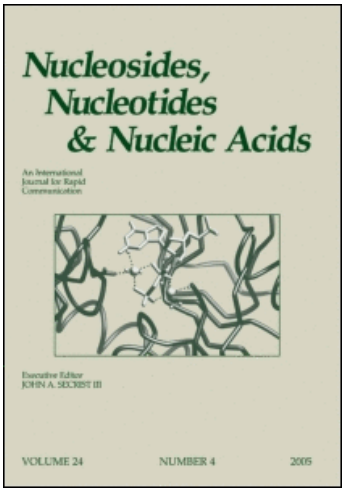

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Design and Synthesis of *bis*-carbamate Analogs of Cyclic *bis*-(3'-5')-Diguanylic Acid (c-di-GMP) and the Acyclic Dimer PGPG

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DESIGN AND SYNTHESIS OF *bis*-CARBAMATE ANALOGS OF CYCLIC *bis*-(3′-5′)-DIGUANYLIC ACID (c-di-GMP) AND THE ACYCLIC DIMER PGPG

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□ *The bacterial second messenger cyclic* bis-*(3′-5′)-diguanylic acid (c-di-GMP) regulates diverse Gram-negative bacterial virulence functions. The pathways that control, or are controlled by, c-di-GMP suggest that c-di-GMP signaling systems may encompass potential drug targets. It is presently undetermined, however, whether up- or down-modulation of c-di-GMP signaling would be the desired therapeutic state. We addressed potential drug target validation by synthesizing non-hydrolysable carbamate analogs of both the cyclic dinucleotide and the acyclic (*seco*) dinucleotide. A molecular docking simulation of the carbamate isostere suggests that this analog is capable of assuming the correct conformation and pose at a c-di-GMP binding site.*

The bacterial second messenger cyclic *bis*-(3′-5′)-diguanylic acid (c-di-GMP), first identified as an activator of cellulose synthase in *Gluconacetobacter xylinus*,[1,2] is currently known to regulate bacterial virulence functions including biofilm formation,[3–6] survival in infected mice, bacterial antioxidant defenses, and killing of host macrophages.[7–9] Signaling from c-di-GMP controls the transition from sessility to motility[6,10] and helps direct the expression of genes responsible for the transition from survival in the external environment—or intermediate host—to survival in the mammalian

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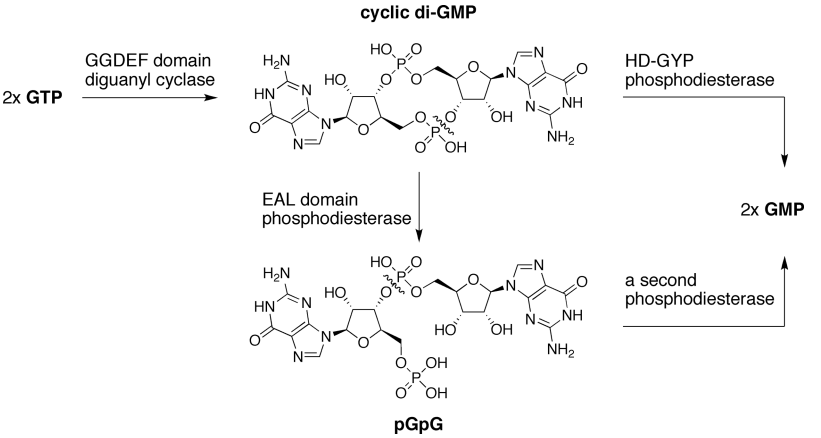


FIGURE 1 The regulation of c-di-GMP is controlled by diguanylcyclases containing a canonical GGDFEF domain and by two alternative classes of phosphodiesterases, those containing a canonical EAL domain and those with an HD-GYP domain. Whereas HD-GYP enzymes degrade c-di-GMP to two molecules of GMP directly, EAL domain enzymes produce the *seco*-dinucleotide pGpG that is subsequently cleaved to GMP by an as-yet-unidentified enzyme.

host.[11] Thus, components of c-di-GMP regulation or the downstream signal transduction cascade may be targets for a novel class of antibiotics.

Intracellular levels of c-di-GMP are tightly regulated (Figure 1) by the formative GGDEF-domain diguanyl cyclases[6,8,12–17] and the degradative EAL-domain,[6,8,18] or HD-GYP-domain,[19] phosphodiesterases. These domains are named for the conserved residues that distinguish them. The HD-GYP domain phosphodiesterases cleave c-di-GMP to two molecules of GMP, whereas the EAL domain phosphodiesterases cleave only one 3′-phosphodiester bond per mole of substrate to give an acyclic intermediate that is subsequently cleaved to GMP by a second esterase. Regulatory proteins having GGDEF and/or EAL domains have been found across a wide array of bacteria, including the significant human pathogens *Yersinia pestis*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar Typhimurium*, *Vibrio cholerae*, *Bordatella pertussis*,[7,9] *Escherichia coli*, and *Chlamydia* species.[9] Within a given species multiple encoded proteins have GGDEF and/or EAL domains, and often both domains are found within a single protein, as illustrated by the genome of *P. aeruginosa* which encodes 17 GGDEF domain-containing, 5 EAL domain-containing, and 16 GGDEF-EAL domain containing sequences.[17] When the two ostensibly opposing domains, GGDEF and EAL, are found within a single protein, it is sometimes observed that one domain is functionally silent but essential for the regulation of the catalytically competent opposing domain.[9] Additional regulation is provided by the binding of the c-di-GMP product to an allosteric inhibitory I-site on the diguanyl cyclase.[18,20]

In addition to regulatory enzymes, several receptor proteins for the downstream signal transduction of c-di-GMP have been identified. A significant number of these receptors contain a conserved PilZ domain;[10,13,21–23] other non-PilZ domain receptors[5] exist as well. Receptors for c-di-GMP are likely to be found beyond the Gram-negative proteome. Although there are no known eukaryotic homologs of the regulatory or receptor proteins, and no eukaryotic production of c-di-GMP, exogenously applied c-di-GMP has immunomodulatory effects on mice.[24–26] Effects against the Gram-positive pathogen *Staphylococcus aureus*, which lacks an endogenous c-di-GMP network, have been reported.[27] The diverse proteins and pathways that control, or are controlled by, c-di-GMP, suggest that c-di-GMP signaling systems may encompass a number of potential drug targets.[11,28] The complexity of the pathways, however, has confounded efforts to determine whether up or down-modulation of c-di-GMP signaling would be the desired therapeutic state.

Approaches to controlling c-di-GMP have been predominantly genetic; the synthetic chemistry applied to this system[2,29–32] has been limited in scope. Published synthetic analogs all retain a phosphoester or phosphothioester bond. We chose to address potential drug target validation within the c-di-GMP signaling matrices by synthesizing nonhydrolysable analogs of both the cyclic dinucleotide and, additionally, of the acyclic (*seco*) dinucleotide. Interest in the *seco* analogs was prompted by the recent suggestion that these intermediate compounds may mediate intracellular effects independently of c-di-GMP.[9,33] Signaling by *seco*-di-GMP is an attractive hypothesis since it rationalizes the existence of the two nonredundant phosphodiesterase enzyme pathways.

We modified the native c-di-GMP structure by replacing the phosphodiester backbone with an isosteric carbamate function[34] that has precedence in antibacterial drugs.[35] Carbamates have been introduced as phosphate surrogates as a strategy to increase both the stability and membrane permeability of the resultant compound.[34] We also deleted the 2′ hydroxyl that has been shown to be nonessential for activity,[2] and, in the *seco* analogs, desymmetrized the dimer in order to expand the repertoire of purines and purine surrogates (Figure 2).

The key intermediate in our strategy to **1** is ribonucleoside **4** that has all heavy atoms in place and protecting/latent functional groups optimized for synthetic flexibility (Scheme 1). Early in our synthetic work we determined that the isobutyryl amide for guanosine afforded good stability during reactions along with good lability to final deprotection conditions. Azido alcohol **4** was converted in a single step to either the protected (**5**) or free (**6**) amino alcohol. Several alternatives were explored for the key carbonyl insertions (Scheme 2). For the first carbamate-forming reaction, attempted activation with either carbonyl diimidazole or *p*-nitrophenyl chloroformate gave no acylation reaction, while phosgene-based activation

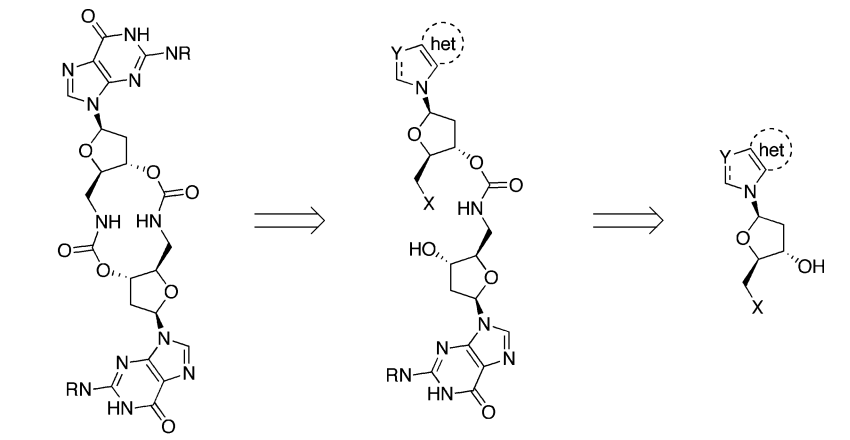


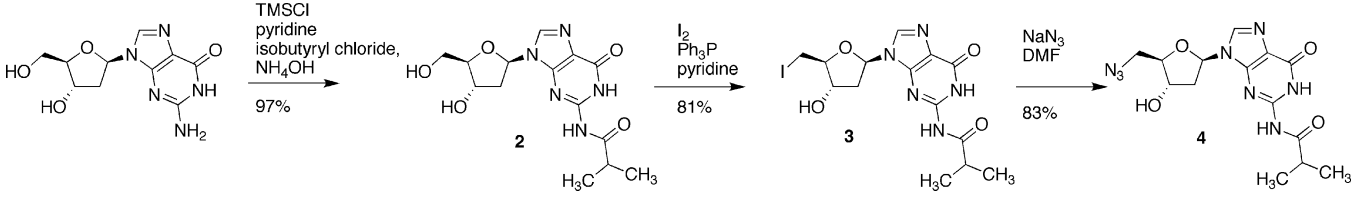
FIGURE 2 Target bicyclic carbamate and *seco* analogs are obtained on the same synthetic pathway.

methods resulted in low yields and generation of impurities. In contrast, activation as the N-hydroxy succinimidyl (NHS) carbonate provided the optimum balance of reactivity and stability to permit coupling to amine **6** in good yield. The second carbamate-forming reaction demonstrated a different profile. Unlike the NHS carbonate of **5**, the **7** NHS carbonate was unstable to purification, whereas reaction with carbonyl diimidazole, followed by in situ N-deprotection, neutralization of the amine, and NHS catalysis, gave **9** in modest yield. The final deprotection to **1** was effected with saturated methanolic ammonia.

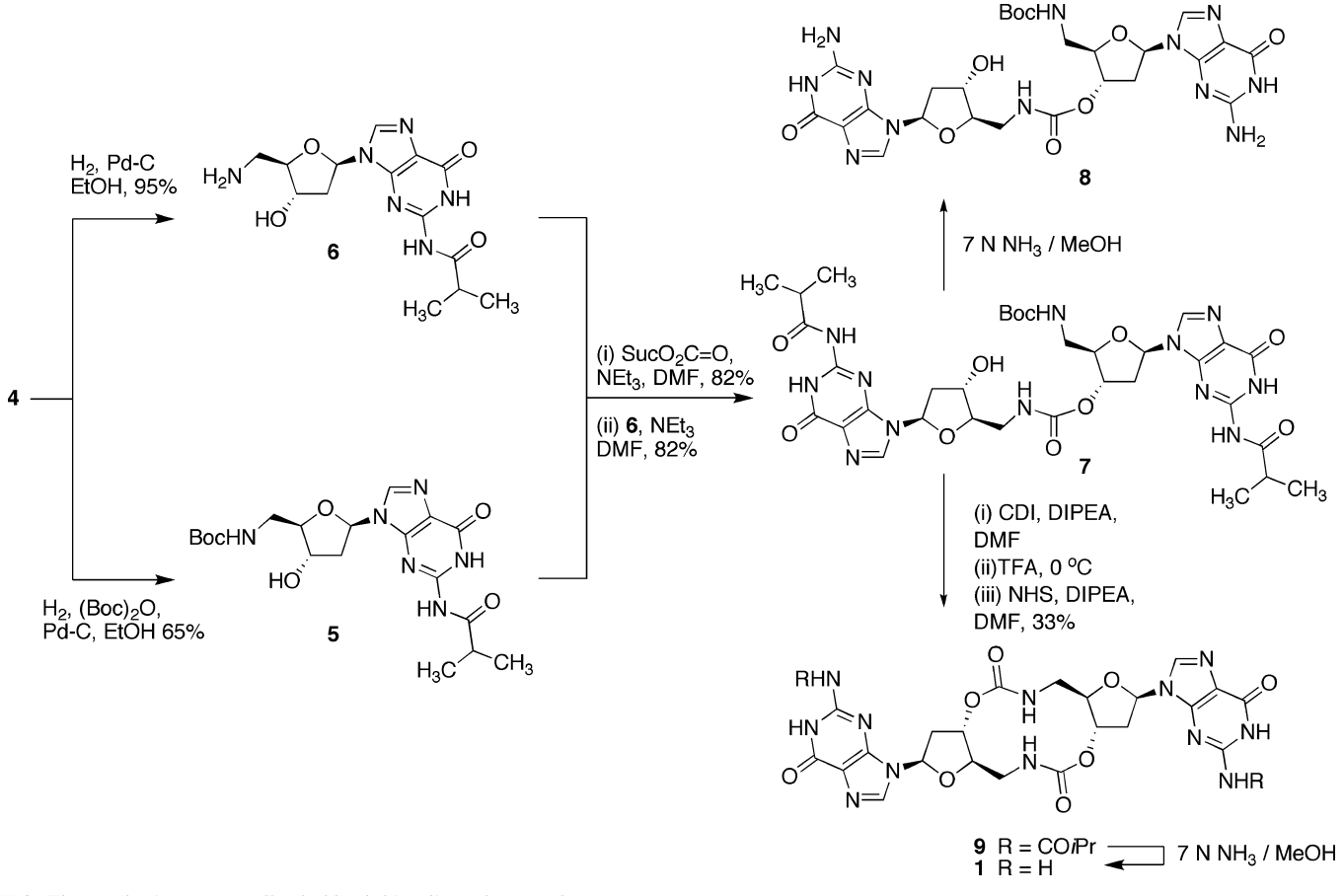
We expanded our panel of *seco* analogs using the methods described in Schemes 1 and 2 to obtain analogs of **8** in which the nucleobase and/or 3′- and 5′-functional groups were varied (Figure 3).

The ensembles of cyclic dinucleotide conformations have been extensively studied.[31] We observed some conformational effects upon the first carbamate formation (compound **7**), and profound effects after the second (compound **9**) carbamoylation. The ^{1}H and ^{13}C spectra of compounds **7–13**, as well as **1**, show broadened peaks with evidence of several aggregate species in solution at 25°C. Upon warming or addition of chaotropic solvents, this aggregation is alleviated, and a single species can be resolved. This is shown for **1** in Figure 4. In the cyclic compounds, aggregation effects can also be observed in the HPLC elution profiles, as has been observed by others,[36] and careful choice of eluent was necessary to maintain and monitor a single species.

A docking study of the *bis*-carbamate **1** gives a structure that closely resembles the crystal structure of native c-di-GMP[37] and c-di-GMP in a co-crystal bound in the allosteric I-site of PleD (Figure 5).[38] The



SCHEME 1 Deoxyriboguanosine was converted to key intermediate **4**.



SCHEME 2 The synthetic strategy afforded both bicyclic and *seco*-carbamates.

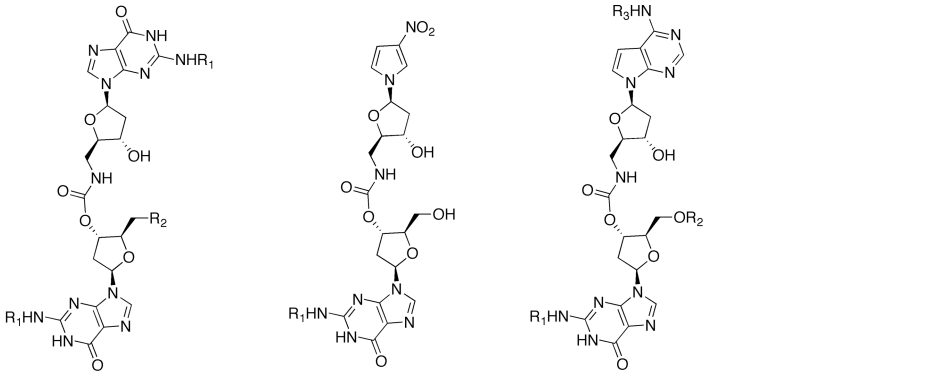


FIGURE 3 The *seco*-carbamate analogs provide structural diversity along the base, sugar, and backbone.

modeled structure of **1** that achieves this conformation has the second best energy of all 25 Monte Carlo docking poses. (The best solution was deemed irrelevant because it did not maintain the internal hydrogen bonds between the two molecules of **1** as observed for the c-di-GMP dimer in the PleD crystal structure; its predicted binding energy was only less than 1 kcal/mol better, beyond the accuracy of empirical binding energy calculations.) The interdigitating C-shapes are similar in orientation for **1** and c-di-GMP, and the hydrogen bond network

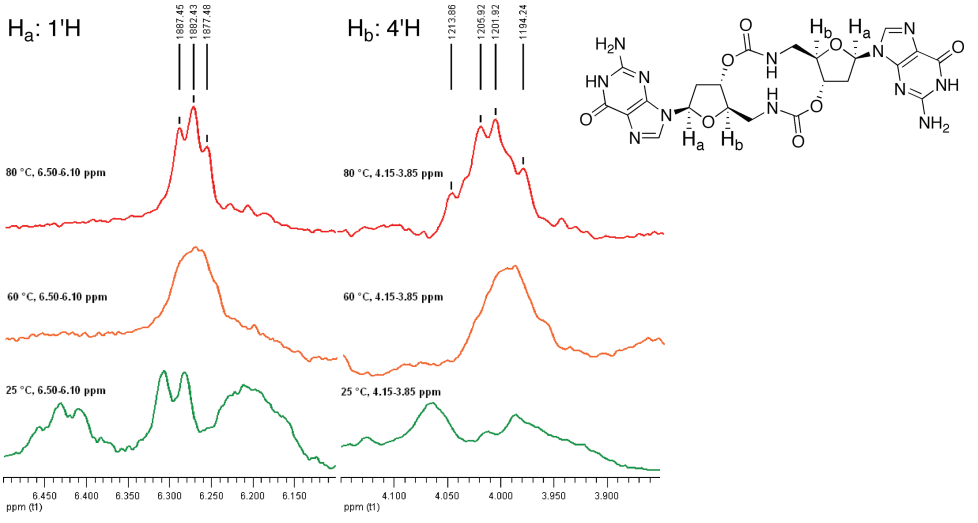


FIGURE 4 ^{1}H NMR resolution of the ribose 1′ and 4′ methines of **1** as a function of temperature. Spectra were recorded in CD_3OD at 300 MHz using a pulse width of 11.50 μsec for 32 transients.

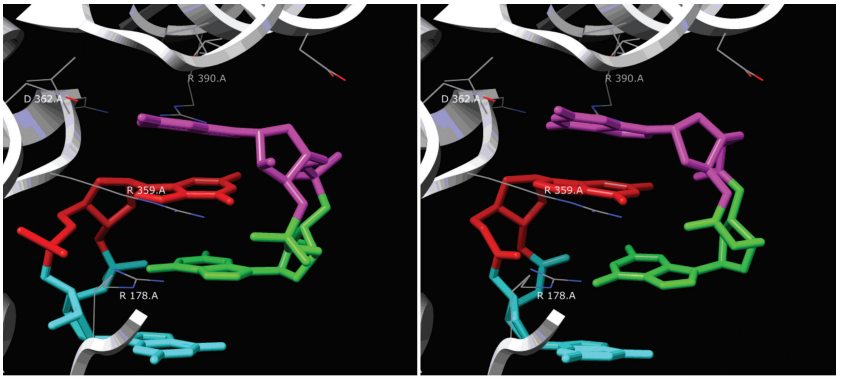


FIGURE 5 Molecular docking of (**A**) c-di-GMP and (**B**) isosteric carbamate analog **1** in the allosteric inhibitory I-site of PleD. The cyclic (covalent) dimeric ligands bind as interdigitating (noncovalent) dimers. For clarity, each cyclic dinucleotide is colored in two colors, one color for each nucleotide moiety. Details of the modeling are given in the Experimental section.

between the two cyclic dinucleotides is preserved. Significantly, 14 out of 17 hydrogen bonds under 3.6 Å between the protein and the ligands are also preserved in the *in silico*-docked carbamate (Figure 6), with the greatest deviation occurring, not surprisingly, at the two of the carbamate oxygens. Interactions of **1** with the PleD I-site residues shown by mutagenesis to be critical for allosteric inhibition, Arg359, Asp362, and Arg390,[18,38] are all among the conserved contacts. Arg359 and Asp362 form the canonical RxxD motif which has been observed in the allosteric inhibitory I-sites across several species' diguanyl cyclases, and Arg390 falls within this site. Upon c-di-GMP binding, this region shows dramatically reduced motility to maintain the c-di-GMP contacts essential to effect product inhibition of the active (A-) site. Six out of seven of the atoms in the ligands that forge these hydrogen bonds are on the guanosines, suggesting a high degree of nucleotide base specificity for inhibition at the I-site. Just as in the c-di-GMP PleD co-crystal, we also observe in our model hydrogen bonds between **1** and Arg148 and 178, and Gly174, Val175, and His177. These residues fall within the Rec2 domain, a noncatalytic domain connected to the GGDEF domain by the I-site.[18]

Whether the conformations predicted for **1**, and available to analogs **7–13**, remain relevant in the physiological setting remains to be experimentally determined. Understanding the biochemical and phenotypic consequences of the isomorphism we have introduced will help address significant questions in c-di-GMP signaling research. With these compounds—the first alternative structures to ribonucleotide ligands—and the synthetic strategies presented in this work, we have in hand the tools to pursue basic questions on this second messenger system.

A

	H-bond between ligands and receptor				
	Ligand Atom	Receptor Atom	Distance (Å) (from crystal structure 1W25, ligands are c-diGMP)	Distance (Å) (After minimization, ligands are carbamate c-diGMP)	Distance (Å) (After Monte Carlo simulation, ligands are carbamate c-diGMP)
1	A1N	D362 OD2	2.9	2.7	2.7
2	A2N	D362 OD1	3.0	2.9	2.9
3	A6O	R390 NH2	2.8	3.0	3.0
4	A7N	R390 NH1	3.3	3.3	3.2
5	A1'O	D383 OD2	3.2	3.3	3.4
6	C6'O	R359 NH2	2.8	2.8	3.0
7	C6O	R178 NH2	3.5	2.9	2.9
8	C7N	R178 NH2	2.6	3.0	4.1
9	B6O	R359 NH2	3.0	2.9	2.8
10	B7N	R359 NE	3.0	3.3	3.6
11	B6'O	R178 NH1	2.7	2.8	3.0
12	B6'OO	A360 N	2.8	Not preserved	Not preserved
13	D3'O	R148 NH1	2.9	3.5	4.5
14	D2'O	R148 NH2	3.3	Not preserved	Not preserved
15	D2N	G174 O	3.1	3.0	3.3
16	D2N	H177 O	3.0	3.0	2.9
17	D2N	V175 O	3.2	3.2	3.2
	H-bond between ligands				
	Ligand Atom	2nd Ligand Atom			
1	A6'O	B1N	2.8	2.8	3.0
2	A6'O	B2N	3.5	3.8	3.3
3	D6'O	C1N	2.9	2.9	3.2
4	D6'O	C2N	3.1	3.4	3.1

B

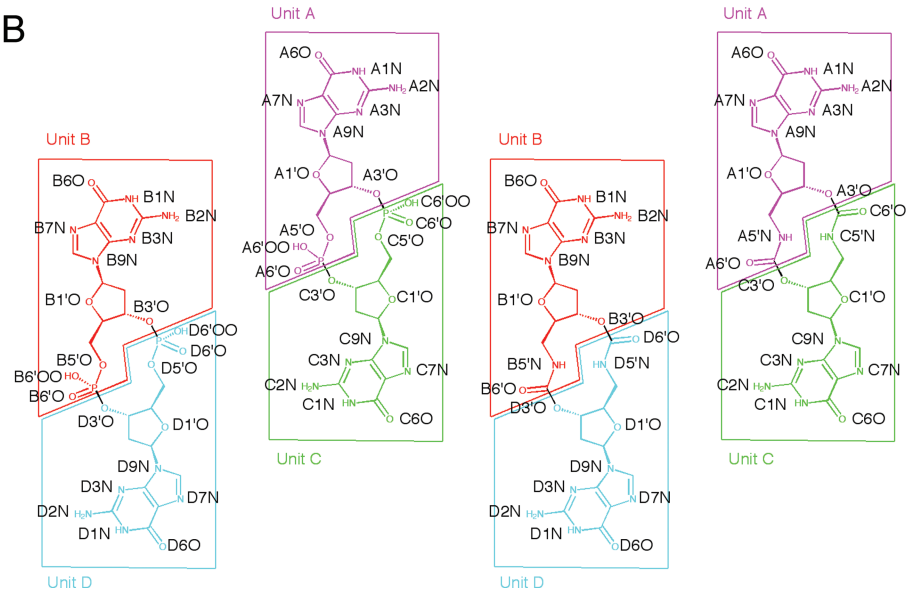


FIGURE 6 (**A**) Protein-ligand and ligand-ligand hydrogen bonds observed in the crystal structure of c-di-GMP/PleD and those predicted from the PleD docking models with c-di-GMP and analog **1**. (**B**) The key to atom numbering for c-di-GMP (left) and analog **1** (right), with colors corresponding to the model in Figure 5.

EXPERIMENTAL

Chemistry

General

All reactions were run under an atmosphere of dry nitrogen. Reagents and solvents were obtained in the highest available purity and used without further purification unless indicated. ^{1}H NMR spectra were obtained on a 300 MHz (Bruker AV300 or AV301) or 500 MHz (Bruker AV500 or Varian) instrument. ^{13}C NMR spectra were obtained on a 500 MHz Bruker AV500. Identity of the compounds was confirmed by mass spectrometry. The compound solution was infused into the electrospray ionization source operating in positive ion mode. Low resolution spectra were obtained on the Esquire LC ion trap mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Accurate mass measurements were performed on the APEX Qe 47 Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics). Normal phase silica gel purifications were done using a Biotage SP4 instrument using the cartridges supplied by Biotage. RP-HPLC was done on a Varian instrument equipped with a diode array ultraviolet detector. For preparative reverse phase chromatography a 10 × 250 mm C18 5 μm column at a flow rate of 4.6 mL/minutes was used; for analytical reverse phase chromatography a 4.6 × 250 mm C18 5μm column at a flow rate of 1 mL/minutes was used. Ultraviolet detection was at 215 and either 254 or 360 nm. Unless otherwise specified, buffer A was 0.05% TFA in H_2O, buffer B was 0.05% TFA in acetonitrile. Thin layer chromatography was done using 0.2 mm polygram SIL G/UV plates (Alltech, Deerfield, IL, USA) or Si250F (J.T. Baker, Phillipsburg, NJ, USA) plates, developed using mobile phases of varying compositions of ethyl acetate/hexane, MeOH/CH_2Cl_2, or MeOH/$CHCl_3$, and visualized by UV light supplemented by vanillin, ninhydrin, and other solution stains where appropriate.

***N*-(9-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)-2-methylpropanamide (2).** 2′-Deoxyguanosine (5.16 g, 19.3 mmol) was suspended in pyridine (20 mL) and the suspension was evaporated. Pyridine (50 mL) was added and the suspension was cooled to 0°C. Trimethylsilyl chloride (12.2 mL, 96.4 mmol) was added by syringe over 5 minutes. 2-Methylpropanoyl chloride (9.36 mL, 96.5 mmol) was added by syringe over 5 minutes and the resulting yellow solution was allowed to warm to room temperature and stir for 3 hours. The solution was cooled to 0°C, water (20 mL) and then ammonium hydroxide (30 mL, 30%) were added and the solution was allowed to warm to room temperature and stir for 1 hours. The pyridine was removed by rotary evaporation, water (50 mL) was added, and the resulting slurry was frozen and dried in vacuo. Water (100 mL) was added, and the residue was dissolved by heating until boiling. The solution was cooled to room

temperature and diethyl ether (20 mL) was added. The resulting layers were cooled to 0°C overnight to form white crystals. Filtration and washing with cold water yielded **2** (6.30 g, 97%) as a white crystalline solid: ^{1}H NMR (CD_3OD, 500 MHz): δ 1.24 (d, 6H, J = 6.9 Hz), 2.43 (m, 1H, J = 6.8, 30.9 Hz), 2.71 (m, 2H), 3.78 (m, 2H, J = 12.0, 3.9 and 26.6 Hz), 4.01 (d, 1H, J = 3.6 Hz), 4.56 (dt, 1H, J = 2.9, 3.4 Hz), 6.39 (t, 1H, J = 6.7 Hz), 8.28 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 19.5, 37.1, 41.9, 63.2, 72.5, 85.7, 89.4, 121.4, 139.7, 149.9, 150.4, 157.7, 181.9; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{19}N_5O_5$, 338.1464; found, 338.1460. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 7.38 minutes.

***N*-(9-((2*R*,4*S*,5*S*)-4-hydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)-2-methylpropanamide (3). 2** (0.484 g, 1.44 mmol) was partially dissolved in pyridine (13 mL). Triphenylphosphene (0.678 g, 2.58 mmol) and iodine (0.548 g, 2.16 mmol) were added and the dark brown solution was stirred under nitrogen for 24 hours. A 10% solution of $Na_2S_2O_3$ (20 mL) was added and the biphasic solution was stirred until the bright yellow color was quenched. The layers were separated and the aqueous layer was washed with $CHCl_3$ (2 × 10 mL). The combined organic layers were washed with brine (40 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography of the residue over silica gel (4 cm × 20 cm), using 0% to 20% MeOH/CH_2Cl_2, gave **3** (0.518 g, 81%) as a cloudy yellow oil: ^{1}H NMR (CD_3OD, 500 MHz): δ 1.23 (d, 6H, J = 10 Hz), 2.45 (m, 1H, J = 3.0, 13.7 Hz), 2.73 (q, 1H, J = 6.9 Hz), 2.87 (q, 1H, J = 6.7 Hz), 3.31 (t, 1H, J = 1.5 Hz), 3.44 (q, 1H, J = 5.5 Hz), 3.51 (q, 1H, J = 6.3 Hz), 4.01 (m, 1H, J = 3.0 Hz), 4.50 (m, 1H, J = 3.1 Hz), 6.36 (t, 1H, J = 6.9 Hz), 8.13 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 7.0, 19.4, 19.5, 19.5, 35.1, 37.0, 40.3, 75.2, 79.6, 85.9, 87.9, 121.6, 131.0, 131.1, 134.1, 134.2, 136.1, 139.8, 149.7, 150.2, 157.5, 157.5, 181.8; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{19}N_5O_4I$, 448.0482; found, 448.0477. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 8.64 minutes.

***N*-(9-((2*R*,4*S*,5*R*)-5-(azidomethyl)-4-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)-2-methylpropanamide (4). 3** (83.5 mg, 0.187 mmol) was dissolved in DMF (2 mL) and NaN_3 was added. The resulting suspension was heated to 50°C overnight. The solvent was removed in vacuo and flash chromatography over silica gel (1 cm × 20 cm), using 5% to 25% MeOH/$CHCl_3$, gave **4** (56.4 mg, 83%) as a white solid: ^{1}H NMR (CD_3OD, 500 MHz): δ 1.23 (d, 6H, J = 6.9Hz), 2.45 (dq, 1H, J = 4.3, 2.9 Hz), 2.73 (m, 1H, J = 6.9 Hz), 2.83 (m, 1H, J = 6.9 Hz), 3.58 (d, 2H, J = 4.9 Hz), 4.06 (q, 1H, J = 4.7 Hz), 4.49–4.52 (m, 1H), 6.35 (t, 1H, J = 6.7 Hz), 8.11 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 19.8, 37.1, 40.4, 53.6, 72.9, 86.0, 87.2, 121.6, 140.4, 181.9; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{18}N_8NaO_4$, 385.1343; found, 385.1355. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 8.17 minutes.

***tert*-butyl ((2*R*,3*S*,5*R*)-3-hydroxy-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methylcarbamate (5).** **4** (195.5 mg, 0.054 mmol) was dissolved in EtOH (8 mL), a solution of di-*tert*-butyldicarbonate (0.810 mL, 1.0 M in THF, 0.810 mmol) and Pd/C (200 mg, 10% by wt) was added. Hydrogen was bubbled through the slurry for 2 hours. The suspension was filtered through celite, rinsing with MeOH. The solvent was removed in vacuo and flash chromatography over silica gel (1 cm × 20 cm), using 5% to 25% MeOH/$CHCl_3$, gave **5**(162.9 mg, 65%) as a white solid:^{1}H NMR (CD_3OD, 500 MHz): δ 1.21 (d, 6H, J = 7.0 Hz), 1.24 (d, 6H, J = 6.9 Hz), 1.58 (s, 9H), 1.60 (s, 9H), 2.38 (d, 1H, J = 5.1 Hz), 2.42 (d, 1H, J = 5.1 Hz), 2.76–2.99 (m, 4H), 3.21–3.28 (m, 4H), 3.66–3.74 (m, 2H), 3.83 (m, 2H, J = 6.6 Hz), 4.28 (s, 2H), 4.54 (s, 2H), 6.29–6.31 (m, 2H), 7.65–7.67 (m, 1H), 7.81 (s, 1H), 9.24 (br, 2H); ^{13}C NMR (CD_3OD, 500 MHz): δ 12.3, 15.3, 19.3, 20.4, 20.5, 20.6, 24.9, 25.8, 31.0, 32.4, 32.6, 37.9, 38.0, 40.6, 41.0, 50.8, 59.2, 69.9, 74.0, 87.4, 130.7, 131.9, 132.0, 133.3, 134.4, 134.9, 135.0, 136.9, 139.3, 150.9, 170.2; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{28}N_6O_6Na$, 459.1968; found, 459.1965. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 11.06 minutes.

***N*-(9-((2*R*,4*S*,5*R*)-5-(aminomethyl)-4-hydroxytetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1*H*-purin-2-yl)-2-methylpropanamide (6).** **4** (178.2 mg, 0.049 mmol) was dissolved in EtOH (5 mL) and Pd/C (110 mg, 10% by wt) was added. Hydrogen was bubbled through the slurry for 2 hours. The suspension was filtered through celite, rinsing with MeOH, and the resulting solution concentrated in vacuo to give **6** (157.2 mg, 95%) as a white solid. ^{1}H NMR (CD_3OD, 500 MHz): δ 1.05 (d, 6H, J = 6.5 Hz), 1.74 (s, 1H), 2.40 (m, 1H, J = 4.5 Hz), 2.60 (m, 1H, J = 7.0 Hz), 2.79 (m, 1H, J = 7.0 Hz), 2.22 (d, 2H, J = 5.5 Hz), 4.08 (s, 1H), 4.59 (s, 1H), 6.21 (t, 1H, J = 6.5 Hz), 7.86 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 19.7, 37.2, 40.2, 50.0, 73.3, 86.6, 125.7, 130.0, 132.6, 138.6, 150.1, 169.4; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{21}N_6O_4$, 337.1619; found, 337.1629. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 6.83 minutes.

***tert*-butyl ((2*R*,3*S*,5*R*)-3-((2,5-dioxopyrrolidin-1-yloxy)carbonyloxy)-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl) methylcarbamate (14).** **5** (101.6 mg, 0.023 mmol) was dissolved in DMF (2 mL) and concentrated in vacuo 3 times to dry the sample. The dry residue was dissolved in DMF (3 mL), triethylamine (50 μL, 0.036 mmol) and N, N′-disuccinimidyl carbonate (120 mg, 0.047 mmol) was added. The reaction was stirred under nitrogen for 18 hours, concentrated in vacuo, and flash chromatography over silica gel (2.5 cm × 20 cm), using 0% to 10% MeOH/$CHCl_3$, gave **14** (110.8 mg, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.19 (d, 6H, J= 6.9 Hz), 1.22 (d, 6H, J = 6.8 Hz), 1.49 (s, 18H), 2.51 (d, 1H, J = 5.0 Hz), 2.56 (d, 1H, J = 5.0 Hz), 2.81 (d, 4H, J = 8.8 Hz), 2.87 (s, 8H), 3.16–3.27 (m, 2H), 3.65–3.75 (m, 2H), 4.39 (s, 2H), 5.27 (d, 2H, J

= 6.2 Hz), 7.69 (s, 2H); ^{13}C NMR (CD_3OD, 500 MHz): δ 18.6, 18.7, 25.4, 28.2, 35.7, 36.4, 43.4, 81.4, 82.9, 83.1, 86.6, 122.8, 138.8, 147.2, 148.9, 151.0, 155.2, 157.8, 168.5, 168.7, 180.2; MS (*m/z*): $[M\text{-}H]^-$ calcd for $C_{24}H_{31}N_7O_{10}$, 576.2; found, 576.2. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 19.23 minutes.

(2*R*,3*S*,5*R*)-2-(azidomethyl)-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-3-yl 2,5-dioxopyrrolidin-1-yl carbonate (15). 4 (23.5 mg, 0.064 mmol) was dissolved in DMF (1 mL) and concentrated in vacuo 3 times to dry the sample. The dry residue was dissolved in DMF (1 mL), triethylamine (10 μL, 0.072 mmol) and N, N′-disuccinimidyl carbonate (17 mg, 0.066 mmol) was added. The reaction was stirred under nitrogen for 18 hours, concentrated in vacuo, and flash chromatography over silica gel (2.5 cm × 20 cm), using 0% to 10% MeOH/$CHCl_3$, gave **15** (16.9 mg, 53%). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.30 (d, 6H, J = 3.5 Hz), 1.31 (d, 6H, J = 3.5 Hz), 2.80–3.02 (m, 2H), 2.81 (s, 4H), 2.91 (s, 4H), 3.76–3.87 (m, 4H), 4.43 (s, 1H), 4.48 (s, 2H), 6.23 (s, 2H), 7.92 (s, 2H), 10.02 (s, 1H), 11.77 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 18.8, 19.0, 25.5, 25.6, 25.7, 36.2, 37.3, 52.2, 82.2, 82.6, 84.2, 119.0, 137.0, 147.4, 148.1, 151.0, 154.2, 168.5, 169.1, 173.7, 180.3; MS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{21}N_9O_8$, 504.2; found, 504.2 $[M+H]^+$. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 14.53 minutes.

***tert*-butyl (2*R*,3*S*,5*R*)-2-(aminomethyl)-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-3-hydroxy-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methylcarbamate (7). 14** (17.4 mg, 0.030 mmol) was dissolved in DMF (1 mL) and concentrated in vacuo 3 times to dry the sample. The dry residue was dissolved in DMF (1 mL), triethylamine (50 μL, 0.072 mmol) and **6** (11 mg, 0.055 mmol) was added. The reaction was stirred under nitrogen for 18 hours, concentrated in vacuo, and flash chromatography over silica gel (2.5 cm × 20 cm), using 0% to 10% MeOH/$CHCl_3$, gave **7** (20.9 mg, 87%). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.40–1.43 (m, 12H), 1.64 (s, 9H), 2.60–2.80 (m, 2H), 2.93–3.0 (m, 1H), 3.09–3.20 (m, 2H), 3.60–3.70 (m, 5H), 4.29 (s, 1H), 4.37 (s, 1H), 4.84 (s, 1H), 5.49 (s, 2H), 6.46–6.51 (m, 2H) 7.38 (br, 1H), 8.19 (s, 1H), 8.29 (s, 2H); ^{13}C NMR (CD_3OD, 500 MHz,): δ 28.0, 28.0, 32.80, 33.9, 37.4, 42.5, 48.2, 87.4, 87.7, 88.0, 92.4, 130,6, 143.5, 143.6, 156.1; MS (*m/z*): $[M+Na]^+$ calcd for $C_{34}H_{46}N_{12}O_{11}Na$, 821.6; found, 821.6. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes, 95% for 5 minutes) retention time: 20.13 minutes.

***iso*-butyryl-protected, 2′-deoxy, carbamate-linked cyclic diGMP (9). 7** (28.8 mg, 0.036 mmol) dissolved in DMF (3 mL), diisopropylethylamine (40 μL, 0.22 mmol) and carbonyldiimidazole (22 mg, 0.14 mmol) added. After stirring for 3 hours, $CHCl_3$ (4 mL) and sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the aqueous layer was washed 4 times with $CHCl_3$ (3 mL), and the collected organic layers were dried and

concentrated. The crust was dissolved in $CHCl_3$ (0.5 mL), the reaction was cooled to 0°C, and trifluoroacetic acid (2 mL) was added. After stirring for 20 minutes, the solution was cooled to -78°C, water (5 mL) was added, and the solution was lyophilized to dryness overnight. The resulting residue was dissolved in DMF (5 mL), and diisopropylethylamine (500 μL, excess) and N-hydroxysuccinamide (10 mg, 0.084 mmol) were added. The reaction was stirred for 3 days, while the disappearance of starting material was monitored by HPLC. Added brine (5 mL) and $CHCl_3$ (10 mL), and the layers were separated. The aqueous layer was rinsed with a mixture of isopropyl alcohol and $CHCl_3$ (20%, 3 × 5 mL), and the combined organic layers were dried and concentrated. Chromatography over silica gel (2.5 cm × 20 cm), using 15% to 35% MeOH/$CHCl_3$, gave **9** (3.7 mg, 33%). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.27 (d, 6H, J = 6.7 Hz), 1.45 (d, 6H, J = 6.6 Hz), 2.90–3.0 (m, 2H), 3.10–3.15 (m, 2H), 4.43–4.50 (m, 2H), 4.67–4.72 (m, 2H), 6.68 (d, 2H, J = 8.6 Hz), 7.10–7.22 (m, 1H), 7.70–7.75 (m, 1H), 8.31 (s, 2H), 8.61 (d, 1H, J = 8.4 Hz), 8.94 (s, 1H); ^{13}C NMR (CD_3OD, 500 MHz): δ 19.5, 30.8, 31.1, 37.2, 37.4, 40.1, 43.1, 71.4, 77.1, 82.6, 86.4, 86.6, 181.9; HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{30}H_{36}N_{12}O_{10}Na$, 747.2575; found, 747.2603. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes, 95% for 5 minutes) retention time 14.74 minutes.

2′-deoxy, carbamate-linked cyclic diGMP (1). **9** (8.7 mg, 0.012 mmol) was dissolved in MeOH (2 mL) and the resulting solution was saturated with ammonia gas. The reaction was heated to 50°C for 2 days, concentrated, and purified by preparative HPLC (5% to 80% H_2O in CH_3CN) to give **1** (1.8 mg, 26%). 1H NMR (CD_3OD, 500 MHz): δ 2.52 (t, 4H, J = 1.7 Hz), 3.88 (br, 4H), 6.12 (br, 2H), 6.39 (br, 4H) 7.76 (s, 2H); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{25}N_{12}O_8$, 585.1918; found, 585.1934. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes) retention time: 8.98 minutes.

(2*R*,3*S*,5*R*)-2-(azidomethyl)-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-3-hydroxy-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methylcarbamate (10a). **15** (8.5 mg, 0.017 mmol) was dissolved in DMF (1 mL) and concentrated in vacuo 3 times to dry the sample. A solution of **15** (11.1 mg, 0.033 mmol) in DMF (1 mL) was added along with $SnBu_2(OAc)_2$ (5 μL, 0.019 mmol). The reaction was stirred for 3 days and then concentrated. Chromatography over silica gel (2.5 cm × 20 cm), using 0% to 30% MeOH/CH_2Cl_2, gave **10a** (12.0 mg, 99%). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.47 (d, 6H, J = 6.7 Hz), 1.50 (d, 6H, J = 6.9 Hz), 2.61–2.69 (m, 2H), 2.84–2.90 (m, 3H) 3.12 (m, 3H, J = 6.7 Hz), 3.85–3.99 (m, 3H), 4.28 (m, 1H), 4.45 (m, 1H), 4.76 (s, 1H), 4.91 (m, 1H, J = 6.8 Hz), 5.48 (s, 1H), 6.56 (s, 2H), 7.54 (s, 1H), 8.32–8.39 (m, 2H); ^{13}C NMR (500 MHz, CD_3OD): δ 17.3, 17.3, 22.2, 23.2, 26.7, 31.9, 38.5, 46.0, 76.8, 81.7, 119.9, 132.8, 145.4; HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{29}H_{36}N_{14}O_9Na$, 747.2687; found,

747.2681. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 13.78 minutes.

(2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-2-(azidomethyl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methylcarbamate (10b). 10a (1.7 mg, 0.0023 mmol) was dissolved in pyridine (1 mL) and 7M ammonia in MeOH (1 mL) was added, the flask was sealed, and the reaction stirred for 24 hours. The residue was purified by preparative HPLC (5% to 70% H_2O in CH_3CN) to give **10b** (1.2 mg, 87%).^{1}H NMR (CD_3OD, 500 MHz): δ 1.94 (s, 2H), 2.30–2.60 (m, 2H), 2.70–2.80 (m, 2H), 3.35 (s, 1H), 3.50–3.60 (m, 3H), 4.10–4.20 (m, 3H), 4.50–4.55 (m, 1H), 5.23 (s, 1H), 5.38 (s, 1H), 6.27–6.37 (m, 2H), 8.15 (s, 2H); ^{13}C NMR (CD_3OD, 500 MHz): δ 15.3, 33.0, 51.1, 52.0, 52.1, 104.0, 158.2, 158.9; MS (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{25}N_{14}O_7$, 585.2; found, 585.2. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 8.48 minutes.

(2*R*,3*S*,5*R*)-2-((dimethoxyphosphorylamino)methyl)-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-3-hydroxy-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methylcarbamate (11a). 10a (7.2 mg, 0.0099 mmol) was dissolved in DMF (1 mL) and trimethylphosphite (100 μL, 0.85 mmol) was added. The reaction was stirred under nitrogen for 18 hours, and then concentrated in vacuo. The residue was purified by preparative HPLC (40% to 60% H_2O in CH_3CN) to give **11a** (2.6 mg, 32%).^{1}H NMR (CD_3OD, 500 MHz): δ 1.21 (d, 12H, J = 3.7 Hz), 1.90–2.02 (m, 2H), 2.79–2.99 (m, 3H), 3.21–3.31 (m, 2H), 3.75–3.80 (m, 6H), 5.26–5.39 (m, 2H), 6.29–6.35 (m, 2H), 8.10–8.22 (br, 2H); ^{31}P NMR (CD_3OD, 500 MHz): δ 16.63; MS (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{44}N_{12}O_{12}$, 807.2939; found, 807.2949. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 15.52 minutes.

(2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-2-((dimethoxyphosphorylamino)methyl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methylcarbamate (11b). 11a (29.0 mg, 0.036 mmol) was dissolved in pyridine (1 mL), 7M ammonia in MeOH was added (1 mL), and the reaction was capped and stirred for 5 days. The unreacted starting material and products were separated by preparative HPLC (5% to 60% H_2O in CH_3CN) to give **11b** (1.9 mg, 8%).^{1}H NMR (CD_3OD, 500 MHz): δ 2.20–2.35 (m, 2H), 2.75 (s, 1H), 3.60–3.68 (m, 6H), 3.91 (s, 2H), 4.09 (s, 2H), 4.34 (s, 2H), 5.22 (s, 2H), 5.82 (br, 2H), 6.35 (br, 1H), 6.74 (s, 2H), 6.90–7.10 (m, 1H), 7.37–7.45 (br, 1H), 7.84–7.90 (m, 2H); ^{31}P NMR (DMSO-d_6, 500 MHz): δ 16.95; HRMS (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{25}N_{14}O_7$, 585.2031; found, 585.2037. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 8.69 minutes.

***tert*-butyl (2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-2-(aminomethyl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-**

3-hydroxytetrahydrofuran-2-yl)methylcarbamate (8). 7 (18.7 mg, 0.023 mmol) was dissolved in pyridine (1 mL), 7M ammonia in MeOH was added (1 mL), and the reaction was capped and stirred for 5 d. The unreacted starting material and products were separated by preparative HPLC (5% to 60% H_2O in CH_3CN) to give **8** (1.1 mg, 9%). ^{1}H NMR (CD_3OD, 500 MHz): δ 2.87 (br, 2H), 3.03 (s, 2H), 3.16 (br, 2H), 3.67–3.77 (m, 4H), 4.42 (s, 2H), 4.92 (s, 1H), 5.54 (s, 1H), 6.59 (br, 2H), 8.50–8.55 (m, 2H); HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{26}H_{34}N_{12}O_9Na$, 681.2469; found, 681.2488. HPLC (CH_3CN:H_2O, 10% to 95% in 20 minutes) retention time: 10.13 minutes.

(2*R*,3*S*,5*R*)-2-(hydroxymethyl)-5-(3-nitro-1*H*-pyrrol-1-yl)tetrahydrofuran-3-yl((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methylcarbamate (12b). (2*R*,3*S*,5*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(3-nitro-1*H*-pyrrol-1-yl)tetrahydrofuran-3-ol (25.7 mg, 0.048 mmol) was dissolved in DMF (1 mL), diisopropylethylamine (50 μL, 0.29 mmol) was added, and the solution was cooled to 0°C. Carbonyldiimidazole (25 mg, 0.15 mmol) was added and the reaction was allowed to warm to 23°C over 2 hours. $CHCl_3$ (6 mL) and $NaHCO_3$ (3 mL) were added and the aqueous layer was rinsed with $CHCl_3$ (3 × 3 mL). The collected organic layers were dried and concentrated in vacuo. The resulting crude residue was dissolved in DMF (1 mL) and cooled to 0°C. Diisopropylethylamine (50 μL, 0.29 mmol) and **6** (20 mg, 0.059 mmol) were added and the solution was warmed to 40°C for 48 hours. The solution was concentrated in vacuo and then dissolved in TFA (10% in $CHCl_3$, 1 mL) and cooled to 0°C. After 2 hours, the reaction was concentrated in vacuo at 0°C, and ammonia (7M in MeOH, 2 mL) was added. After stirring for 4 d, the solution was concentrated in vacuo and purified by preparative HPLC (5% to 60% H_2O in CH_3CN) to give **12b** (0.75 mg, 5% over 4 steps). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.63–1.72 (m, 2H), 2.20–2.25 (m, 1H), 2.00–2.10 (m, 1H), 2.68 (s, 1H), 2.89 (s, 1H), 3.48 (s, 2H), 3.55–3.64 (m, 3H), 3.67–3.76 (m, 1H), 4.20–4.23 (m, 1H), 4.37 (br, 2H), 7.64–7.74 (m, 2H), 7.94–8.09 (m, 3H); ^{13}C NMR (CD_3OD, 500 MHz): δ 8.6, 18.6, 18.7, 35.8, 45.9, 53.9, 55.2, 63.8, 72.7, 84.5, 86.8, 87.5, 88.8, 105.9, 113.1, 119.4, 119.5, 120.4, 127.0, 127.9, 128.0, 128.1, 130.0, 130.1, 131.0, 133.8, 135.2, 135.4, 137.2, 155.8, 157.3, 158.6; HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{20}H_{24}N_8O_9Na$, 543.1564; found, 543.1564. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes, 95% for 5 minutes) retention time: 7.19 minutes.

(2*R*,3*S*,5*R*)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(4-phenylamido-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-3-hydroxy-5-(2-(2-methylpropanamido)-6-oxo-1*H*-purin-9(6*H*)-yl)tetrahydrofuran-2-yl)methylcarbamate (13a). *N*-(7-((2*R*,4*S*,5*R*)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)benzamide (21.4 mg, 0.033 mmol) was dissolved in DMF (1 mL), diisopropylethylamine (50 μL, 0.29 mmol) was added, and the solution was cooled to 0°C. Carbonyldiimidazole (18 mg, 0.11 mmol)

was added and the reaction was allowed to warm to 23°C over 2 hours. $CHCl_3$ (6 mL) and brine (3 mL) were added and the aqueous layer was rinsed with $CHCl_3$ (3 × 3 mL). The collected organic layers were dried and concentrated in vacuo. The resulting crude residue was dissolved in DMF (1 mL) and cooled to 0°C. Diisopropylethylamine (50 μL, 0.29 mmol) and **6** (20 mg, 0.059 mmol) were added and the solution was warmed to 40°C for 48 hours. The solution was concentrated in vacuo and purified by preparative HPLC (5% to 60% H_2O in CH_3CN) to give **13a** (12.03 mg, 36% over 2 steps). ^{1}H NMR (CD_3OD, 500 MHz): δ 1.40 (t, 6H, J = 7.3 Hz), 2.30–2.40 (m, 1H), 2.60–2.65 (m, 1H), 3.11 (q, 4H, J = 7.3 Hz), 3.45–3.50 (m, 1H), 3.78 (s, 2H), 4.35–4.45 (m, 1H), 5.54 (d, 1H, J = 4.3 Hz), 6.25–6.30 (m, 1H), 6.81 (d, 2H, J = 8.1 Hz), 7.30–7.55 (m, 4H), 7.87 (s, 1H), 8.02 (d, J = 7.4 Hz, 1H), 9.51 (br, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 8.6, 18.7, 35.7, 37.9, 43.6, 45.9, 55.2, 64.0, 72.8, 83.4, 83.7, 86.5, 86.7, 87.4, 105.0, 109.0, 113.1, 121.2, 123.5, 127.0, 127.7, 127.8, 128.2, 128.9, 130.1, 134.7, 135.4, 139.3, 144.4, 157.5, 158.5; HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{54}H_{54}N_{10}O_{11}Na$, 1041.3871: found, 1041.3861. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes, 95% for 5 minutes) retention time: 13.88 minutes.

(2*R*,3*S*,5*R*)-5-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl ((2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methylcarbamate (13c). 13a (9.5 mg, 0.0093 mmol) dissolved in TFA (10% in $CHCl_3$, 1 mL) and cooled to 0°C. After 1 hour, the reaction was concentrated in vacuo at 0°C, and then ammonia (7M in MeOH, 2 mL) was added. After stirring for 4 days, the solution was concentrated in vacuo and purified by preparative HPLC (5% to 60% H_2O in CH_3CN) to give **13c** (0.8 mg, 16% over 2 steps) and **13b** (1.0 mg, 17% over 2 steps).**13c**:^{1}H NMR (CD_3OD, 500 MHz): δ 2.27 (s, 2H), 2.73 (br, 2H), 3.51–3.61 (m, 1H), 3.72–3.78 (m, 1H), 5.30–5.35 (m, 2H), 6.53 (br, 3H), 7.10 (br, 1H), 7.28 (br, 1H), 7.44 (br, 1H), 7.40–7.60 (m, 1H), 7.90–8.10 (m, 2H); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{27}N_{10}O_7$, 543.2064; found, 543.2072. **13b:** ^{1}H NMR (CD_3OD, 500 MHz): δ 2.20–2.30 (m, 1H), 2.76 (d, J = 8.7 Hz, 2H), 3.67 (d, 2H, J = 3.5 Hz), 3.95–4.08 (m, 2H), 4.36 (s, 1H), 5.31 (s, 1H), 6.16 (s, 1H), 6.34 (s, 1H), 6.70–6.80 (m, 2H), 7.46–7.55 (m, 6H), 7.75–8.10 (m, 3H), 8.60 (s, 1H), 10.55 (s, 1H), 10.93 (s, 1H); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{29}N_{10}O_8Na_2$, 691.1965; found, 691.1951. HPLC (CH_3CN:H_2O, 10% to 95% in 10 minutes, 95% for 5 minutes) retention time: 7.64 minutes.

Molecular Modeling

Molecular docking studies were carried out using QXP/FLO[39,40] and the crystal structure of c-diGMP bound to PleD from *Caulobacter crescentus* (PDB 1W25). The carbamate analog **1** was modeled by building the carbamate group onto the crystallographically observed conformation of

c-di-GMP. Polar hydrogens were added to protein and ligand atoms, and water molecules were removed. The active sites were defined as residues within 7.0 Å of c-di-GMP. Protein atoms were fixed during docking. Two distance constraints to mimic the internal hydrogen bonds observed in the bound c-di-GMP were set up for docking: A6′O—B1N and D6′O—C1N. Docking of **1** was carried out using 2,000 cycles of Metropolis Monte Carlo conformational searching followed by energy minimization. The 25 lowest energy ligand poses were visually inspected.

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