SUPPORTING INFORMATION

Identification of c-di-GMP derivatives resistant to an EAL domain phosphodiesterase

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Activity of CC3396 after 24 hour incubation at room temperature. Degradation of dithiophosphate analogs was analyzed after incubation periods as long as 24 hours at room temperature. To ensure that the phosphodiesterase protein was still active after this time period, we incubated CC3396 (1 μM) in reaction buffer (NaCl, Tris pH 8.0, and $MgCl_2$) for 24 hours before adding c-di-GMP. c-di-GMP (100 μM) was then added and incubated with the protein for 15 minutes before the reaction was quenched and analyzed by HPLC. HPLC analysis indicates that c-di-GMP is completely converted to pGpG even after the protein was pre-incubated at room temperature for 24 hours (Figure S3). This indicates that the protein retains activity and that lack of degradation of these phosphate modified analogs is not due to a loss of protein activity.

TABLES

Table S1. Sequence alignments of select structurally and/or biochemically characterized EAL domain proteins.

Protein[a]	Conserved Residues											Active?
TBD1265	E523	R527	N584	E616	D646	D647	K667	E703	E706[b]	Q509[b]	N725[b]	Yes
CC3396	E	R	N	E	D	D	K	E	E	Q	Y	Yes
BlrP1	E	R	N	E	D	D	K	E	E	Q	F	Yes
RocR	E	R	N	E	D	D	K	E	E	Q	Y	Yes
FimX	E	R	H	Q	S	Q	K	P	E	Q	Y	No
LapD	K	R	N	E	Q	R	K	E	E	Q	Q	No
Residue Role[c]	M1	P2	M1 P1	M1	M1 M2	M2 W2	W1	M2	G2	G2	G2	

[a]Sequences aligned relative to the active EAL domain protein TBD1265 from *T. denitrificans*. Other EAL proteins aligned include CC3396 from *C. crescentus* (the PDE used in this study), BlrP1 from *Klebsiella pneumoniae,* RocR from *Pseudomonas aeruginosa*, FimX from *Pseudomonas aeruginosa*, and LapD from *Pseudomonas fluorescens.*

[b] Previously published work demonstrated that mutation of these residues to alanine did not significantly affect catalysis(Tchigvintsev et al. 2010).

[c] Predicted role of residue in c-di-GMP binding and/or catalysis based on structural characterization of TBD1265 and BlrP1(Tchigvintsev et al. 2010; Barends et al. 2009). M1= metal 1, M2= metal 2, P1= scissile phosphate, P2= phosphate distal to the site of catalysis, W1= nucleophilic water molecule, W2= water molecule 2, and G2= guanine base 2 (refer to Figure 1b,c).

Table S2. Characterization of dithiophosphate analogs after treatment with the PDE CC3396 for 24 hours. The observed mass corresponds to the mass of the cyclic molecule indicating that the linear product is not being formed and co-eluting with the cyclic compound.

		Observed Mass (negative ion)
Analog	**Exact Mass**	**[M-H]/1**
c-(R_PR_P)-di-G_{ps}	722.05	721.0426
c-(R_PS_P)-di-G_{ps}	722.05	721.0412

FIGURES

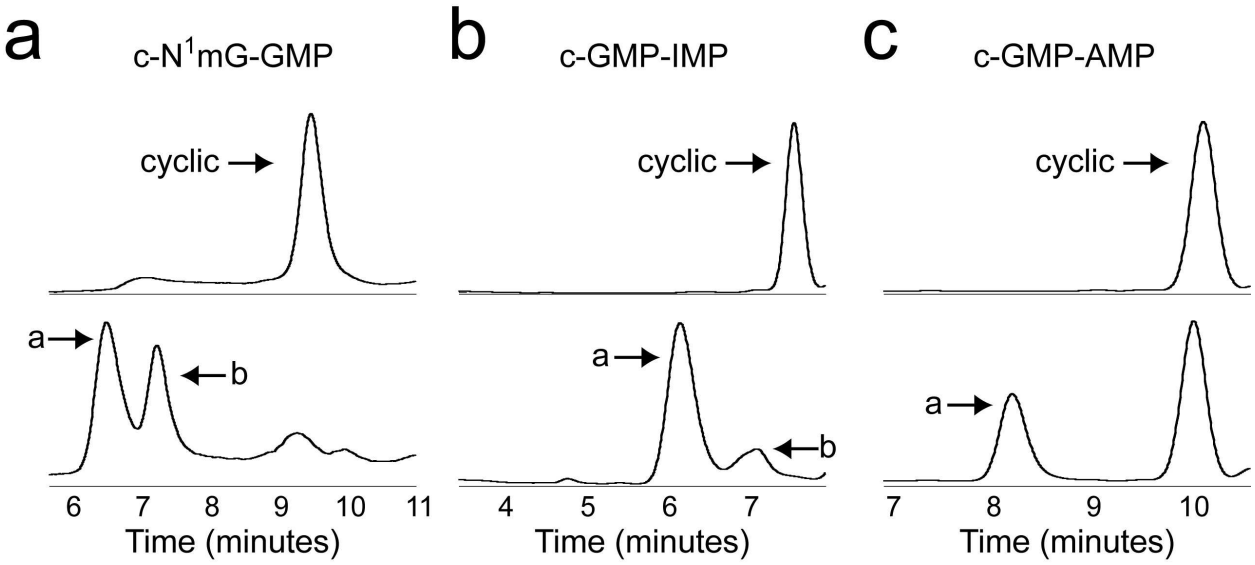

Figure S1. Degradation analysis of analogs containing a single base substitution. Elution profile of untreated analog (top panel) and elution profile of analogs after treatment with CC3396 (bottom panel). (a) c-N^1mG-GMP elutes at 9.5 minutes whereas 2 new peaks are observed after treatment with CC3396 (peak a, RT= 6.8 minutes; peak b, RT= 7.5 minutes). (b) c-GMP-IMP elutes at 7.5 minutes. In the presence of protein, a major product peak (peak a, RT= 6.1 minutes) and minor product peak (peak b, RT= 7 minutes) are observed. (c) c-GMP-AMP elutes at 10 minutes, however only a single product peak (peak a, RT=8.2 minutes) is observed upon degradation of the cyclic compound.

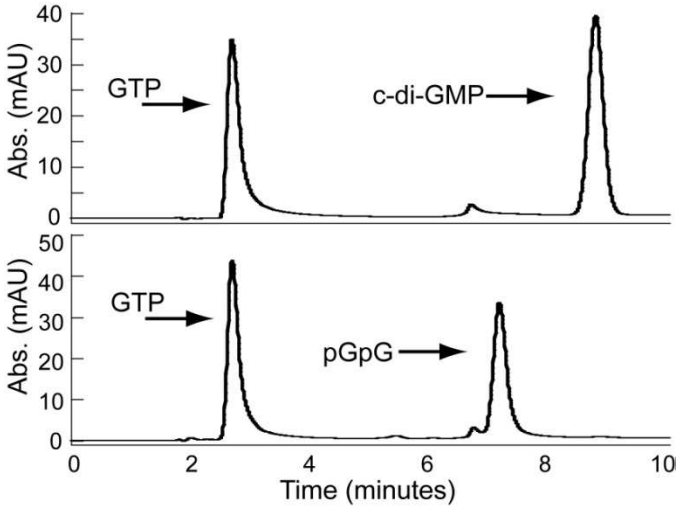

Figure S2. Analysis of the activity of CC3396 after 24 hours. c-di-GMP in reaction buffer in the absence of protein (top) and product analysis after incubating c-di-GMP with protein. c-di-GMP is completely converted to pGpG even after the protein has been kept at room temperature for 24 hours.