

# 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™

Bisphosphorothioate analog of c-di-AMP, Rp isomers

Catalog # vac-nacda2r

For research use only. Not for use in humans.

Version # 17A11-MM

## PRODUCT INFORMATION

### Content:

- 500 µg of lyophilized 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™
- 10 ml sterile endotoxin-free physiological water (NaCl 0.9%)

### Storage and stability:

- 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™ is shipped at room temperature and should be stored at -20 °C. Lyophilized product is stable for 1 year when properly stored.
- Upon resuspension, prepare aliquots of 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™ and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

### Quality control:

- 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™ is a preclinical grade preparation of the cyclic dinucleotide 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp). It is prepared under strict aseptic conditions and is tested for the presence of endotoxins. 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™ is guaranteed sterile and its endotoxin level is <0.005 EU/µg.
- Purity and structure has been determined by LC/MS and NMR: ≥ 95%
- Biological activity has been assessed by measuring induction of the interferon pathway in THP1-Blue™ ISG cells.

## METHODS

### Preparation of sterile stock solution (1 mg/ml):

- Before opening the vial, centrifuge it briefly and open the lid carefully to avoid any loss of product.
- Add 500 µl of sterile endotoxin-free physiological water to 500 µg of 2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) VacciGrade™ to obtain a stock solution at 1 mg/ml.
- Vortex until completely dissolved.

**Working Concentration:** 5-50 µg/mouse

## CHEMICAL PROPERTIES

**Synonym:** (R,R)-(2',3')c-diAM(PS)<sub>2</sub>, (2',3')-Rp,Rp-c-diAMPSS

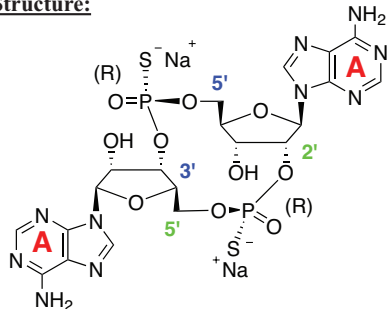
**CAS number:** 1638241-89-0

**Formula:** C<sub>20</sub>H<sub>22</sub>N<sub>10</sub>O<sub>10</sub>P<sub>2</sub>S<sub>2</sub>•2Na

**Molecular weight:** 734.50

**Solubility:** 50 mg/ml in physiological water

### Structure:



## DESCRIPTION

2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) is the Rp,Rp-isomer of the 2'3' bisphosphorothioate analog of 3'3'-cyclic adenosine monophosphate (c-di-AMP). c-di-AMP is second messenger molecule produced by bacteria that has potent immunostimulant activity in mammals<sup>1</sup>. This cyclic dinucleotide (CDN) induces the production of type I interferons (IFNs) following its recognition by the endoplasmic reticulum-resident receptor STING (stimulator of interferon genes) and the recruitment of TBK1 (TANK-binding kinase 1) and IRF3 (interferon regulatory factor 3)<sup>2</sup>.

2'3'-c-di-AM(PS)<sub>2</sub> (Rp,Rp) has a higher affinity for STING than c-di-AMP due to the presence of a 2'-5', 3'-5' mixed linkage, as found in endogenous human CDNs produced by cGAS (cyclic GMP-AMP (cGAMP) synthase)<sup>3</sup>. It activates all known human STING alleles as well as mouse STING. In addition, this analog contains two phosphorothioate diester linkages to protect it against degradation by phosphodiesterases that are present in host cells or in the systemic circulation<sup>4</sup>. The Rp, Rp dithio diastereoisomer was found to induce higher type I IFN production compared to the Rp/Sp dithio diastereoisomers or c-di-AMP<sup>3</sup>.

InvivoGen has developed STING reporter cells in two well established immune cell models: human THP-1 monocytes and mouse RAW 264.7 macrophages. These cells express a reporter gene (SEAP or Lucia luciferase) under control of an IRF-inducible and/or NF-κB-inducible promoter.

1. Woodward JJ. *et al.*, 2010. c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. *Science*.328(5986):1703-5. 2. Jin L. *et al.*, 2011. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J Immunol.* 187(5):2595-601. 3. Corrales L *et al.*, 2015. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Rep.* 11(7):1018-30. 4. Yan H. *et al.*, 2008. Synthesis and immunostimulatory properties of the phosphorothioate analogues of cdiGMP. *Bioorg. Med. Chem. Lett.* 18, 5631–5634.

## RELATED PRODUCTS

Product	Description	Cat. Code
2'2'-cGAMP VacciGrade™	STING ligand	vac-nacga22
2'3'-cGAMP VacciGrade™	STING ligand	vac-nacga23
3'3'-cGAMP VacciGrade™	STING ligand	vac-nacga
AddaVax™	Squalene-Oil-in-water	vac-adx-10
Alhydrogel® 2%	Al(OH) <sub>3</sub> gel	vac-alu-250
c-di-AMP VacciGrade™	STING ligand	vac-nacda
CFA	Complete Freund's adjuvant	vac-cfa-10
EndoFit™ Ovalbumin	For <i>in vivo</i> use	vac-pova
Flagellin FlIC VacciGrade™	TLR5 ligand	vac-fla
MPLAs VacciGrade™	TLR4 ligand	vac-mpls
ODN 2006 VacciGrade™	Human TLR9 ligand	vac-2006-1
Poly(I:C) VacciGrade™	TLR3 ligand	vac-pic

### TECHNICAL SUPPORT

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