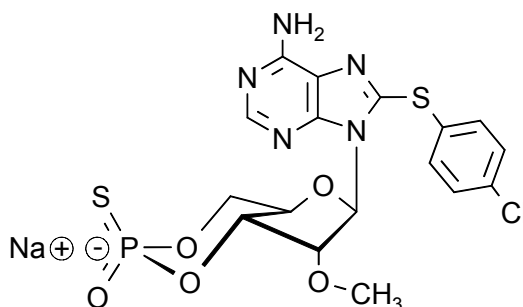


Technical Information about 8- (4- Chlorophenylthio)- 2'- O- methyl- cAMPS, Sp- isomer

Specific, membrane-permeant and metabolically stable activator of the Epac cAMP receptor

Update: May 31, 2011 AI



Abbreviation: Sp-8-pCPT-2'-O-Me-cAMPS / Sp-8-CPT-2'-O-Me-cAMPS

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
C ₁₇ H ₁₆ ClN ₅ O ₅ PS ₂ ·Na	[634208-37-0]	523.9	λ _{max} 282 nm / ε 16000 / pH 7	C 052

Name: 8-(*para*-Chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphorothioate, Sp-isomer (Sp-8-pCPT-2'-O-Me-cAMPS) or 8-(4-chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphorothioate, Sp-isomer (Sp-8-CPT-2'-O-Me-cAMPS)

Legal information: The Reagent is subject to patent application n° EP 02077219.0 and foreign equivalents. The Reagent and any improvements are owned and licensed by The UMCU/ UMCU Holding Pharmaceuticals. The Reagent is sold under limited, non transferable and non exclusive licence from The UMCU/ UMCU Holding for research purposes only, to the exclusion of any commercial use, transfer or otherwise sale of this Reagent or its components or derivatives to a third party. Use or sale of Reagent for any commercial purposes requires a commercial license from The UMCU - Universiteitsweg 100, Utrecht, The Netherlands / UMCU Holding - Yalelaan 40, Utrecht, The Netherlands.

Description: Sp-8-pCPT-2'-O-Me-cAMPS is an analogue of the natural signal molecule cyclic AMP in which the hydrogen in position 8 of the heterocyclic nucleobase is replaced by the lipophilic 4-chlorophenylthio moiety, and the ribose 2'-hydroxyl group has been methylated. In addition, the axial one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is modified by sulfur. The suffix "p" indicates that R/S nomenclature refers to phosphorus.

Properties: Sp-8-pCPT-2'-O-Me-cAMPS is a hydrolysis-resistant form of 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041) which is a potent stimulator of the exchange factors directly activated by cAMP (Epac or cAMP-GEF). Since a free 2'-ribose hydroxyl group in cyclic AMP is essential for stimulation of cAMP-dependent protein kinase (PKA), the methylated structure of Sp-8-pCPT-2'-O-Me-cAMPS is an extremely poor PKA activator and allows for specific discrimination between both signalling pathways. On the other hand, potent activators of PKA carrying a modified 6 position at the adenine nucleobase can be used as Epac-negative controls. N⁶-modified cyclic AMP analogues such as N⁶-Benzoyl-cAMP (Cat. No. B 009) or N⁶-Phenyl-cAMP (Cat. No. P 006) are specific PKA agonists, but show only neglectable agonistic properties on Epac.

The high lipophilicity of Sp-8-pCPT-2'-O-Me-cAMPS (> dibutyryl-cAMP) allows for good membrane permeability in most biosystems, and its resistance towards phosphodiesterases prevents from hydrolysis.

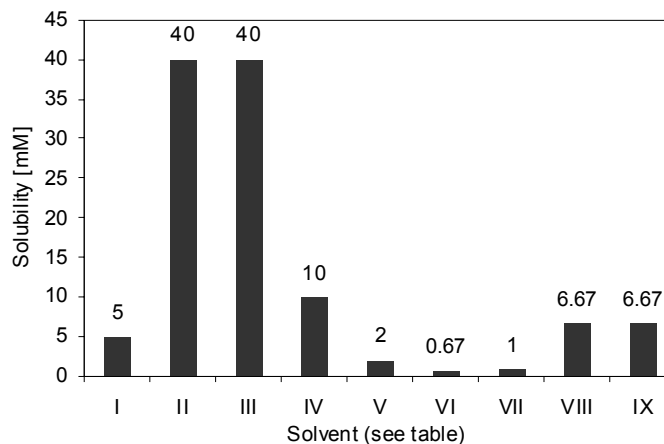
Specification: Lyophilized or crystallized sodium salt. The free acid or other salt forms of Sp-8-pCPT-2'-O-Me-cAMPS are available upon request. **Equal concentrations of Sp-8-pCPT-2'-O-Me-cAMPS can appear very different in volume due to sensitivity of the lyophilized form to humidity. The compound can even contract to small volume droplets.** Normally the product is located in the conical bottom of the tube. Micromolar quantities are determined by UV at λ_{max}.

Purity: Typical analysis is better than 99% (HPLC / UV / 282 nm). The product is not sterile and has not been tested for endotoxins.

Stability and Storage: Sp-8-pCPT-2'-O-Me-cAMPS is chemically rather stable. Nevertheless, we recommend that the compound should be stored in the freezer, for longer storage periods preferably in freeze-dried form. Since UV radiation develops a fluorescent impurity, which can disturb in fluorescence assays, avoid bright light during handling and experiments.

Solubility: Detailed information on the solubility of Sp-8-pCPT-2'-O-Me-cAMPS in water and various buffers are listed in the solubility chart below. Concentrations have been determined at ambient temperature and can be considered as minimum concentrations usually obtainable, however, slight batch-to-batch variations cannot be ruled out. When opening the tube please make sure that no substance is lost within the cap. Please rinse tube walls carefully and preferably use ultrasonic or vortex to achieve total and uniform mixing.

No.	Solvent	Solubility [mM]
I	H ₂ O	5
II	DMSO	40
III	DMF	40
IV	Ethanol 96%	10
V	Methanol	2
VI	PBS, pH 7.4	0.67
VII	100 mM Na ₂ HPO ₄ , pH 7.0	1
VIII	25 mM HEPES/NaOH, pH 7.2	6.67
IX	25 mM Tris/HCl, pH 7.4	6.67



Toxicity and Safety: Since cyclic AMP has multiple tasks in every organism it is very likely that lipophilic cAMP analogues will interfere with many cell regulation processes *in vivo*. However, due to the rather small quantities to work with no health hazards have been reported. Nevertheless please keep in mind that the *in vivo* properties of this compound are not sufficiently characterized up to now. Avoid skin contact or ingestion and allow only trained personnel to handle the product. Our products are designed, developed and sold for research purposes only. They are intended for *in vitro* and nonhuman *in vivo* laboratory applications. Any other use requires approval of health authorities.

Not for drug, household or related uses!

Selected References for Sp-8-pCPT-2'-O-Me-cAMPS:

- Dzhura, I.; Chepurny, O.G.; Kelley, G.G.; Leech, C.A.; Roe, M.W.; Dzhura, E.; Afshari, P.; Malik, S.; Rindler, M.J.; Xu, X.; Lu, Y.; Smrcka, A.V.; Holz, G.G., *J. Physiol.*, **588**, 4871 - 4889 (2010): "EPAC2-dependent Mobilization of Intracellular Ca²⁺ by Glucagon-like Peptide-1 Receptor Agonist Exendin-4 is Disrupted in Beta-Cells of Phospholipase C-Epsilon Knockout Mice"
- Waidmann, O.; Pleli, T.; Dvorak, K.; Baehr, C.; Mondorf, U.; Plotz, G.; Biondi, R.M.; Zeuzem, S.; Piiper, A., *J.Biol.Chem.*, **284**, 32256 - 32263 (2009): "Inhibition of the Equilibrative Nucleoside Transporter 1 and Activation of A2A Adenosine Receptors by 8-(4-Chlorophenylthio)-modified cAMP Analogs and their Hydrolytic Products"
- Poppe, H.; Rybalkin, S.D.; Rehmann, H.; Hinds, T.R.; Tang, X.-B.; Christensen, A.E.; Schwede, F.; Genieser, H.-G.; Bos, J.L.; Doeskaland, S.O.; Beavo, J.A.; Butt, E., *Nature Methods*, **5**, 277 - 278 (2008): "Cyclic Nucleotide Analogs as Probes of Signaling Pathways"
- Yokoyama, U.; Patel, H.H.; Lai, N.C.; Aroonsakool, N.; Roth, D.M.; Insel, P.A., *PNAS*, **105**, 6386 - 6391 (2008): "The cyclic AMP Effector Epac Integrates Pro- and Anti-fibrotic Signals"
- Ouyang, M.; Zhang, L.; Zhu, J.; Schwede, F.; Thomas, S.A., *PNAS*, **105**, 11993 - 11997 (2008): "Epac Signaling is Required for Hippocampus-dependent Memory Retrieval"
- Kang, G.; Leech, C.A.; Chepurny, O.G.; Coetzee, W.A.; Holz, G.G., *J. Physiol.*, **587**, 1307 - 1319 (2008): "Role of the cAMP Sensor Epac as a Determinant of K_{ATP} Channel ATP sensitivity in Human Pancreatic β -cells and Rat INS-1 Cells"
- O'Neill, J.S.; Maywood, E.S.; Chesham, J.E.; Takahashi, J.S.; Hastings, M.H., *Science*, **320**, 949 - 953 (2008): "cAMP-Dependent Signaling as a Core Component of the Mammalian Circadian Pacemaker"
- Harrisingh, M.C.; Nitabach, M.N., *Science*, **320**, 879 - 880 (2008): "Integrating Circadian Timekeeping with Cellular Physiology"
- Haag, S.; Warnken, M.; Juergens, U.R.; Racké, K., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **378**, 617 - 630 (2008): "Role of Epac1 in Mediating Anti-proliferative Effects of Prostanoid EP2 Receptors and cAMP in Human Lung Fibroblasts"
- Holz, G.G.; Chepurny, O.G.; Schwede, F.S., *Cell. Signal.*, **20**, 10 - 20 (2007): "Epac-selective cAMP Analogs: New Tools with which to Evaluate the Signal Transduction Properties of cAMP-regulated Guanine Nucleotide Exchange Factors"
- Laxman, S.; Riechers, A.; Sadilek, M.; Schwede, F.; Beavo, J.A., *PNAS*, **103**, 19194 - 19199 (2006): "Hydrolysis Products of cAMP Analogs Cause Transformation of Trypanosoma Brucei from Slender to Stumpy-like Forms"

Selected References for 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041):

For an extended list please inquire or refer to our website www.biolog.de.

- Rehmann, H.; Schwede, F.; Doeskaland, S.O.; Wittinghofer, A.; Bos, J.L., *J. Biol. Chem.*, **278**, 38548 - 38556 (2003): "Ligand-mediated Activation of the cAMP-responsive Guanine Nucleotide Exchange Factor Epac"

Christensen, A.E.; Selheim, F.; de Rooij, J.; Dremier, S.; Schwede, F.; Dao, K.K.; Martinez, A.; Maenhaut, C.; Bos, J.L.; Genieser, H.-G.; Døskeland, S.O., *J. Biol. Chem.*, **278**, 35394 - 35402 (2003): "cAMP Analog Mapping of Epac1 and cAMP-Kinase. Discriminating Analogs Demonstrate that Epac and cAMP-Kinase Act Synergistically to Promote PC-12 Cell Neurite Extension"

Kang, G.; Joseph, J.W.; Chepurny, O.G.; Monaco, M.; Wheeler, M.B.; Bos, J.L.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *J. Biol. Chem.*, **278**, 8279 - 8285 (2003): "Epac-selective cAMP Analog 8-pCPT-2'-O-Me-cAMP as a Stimulus for Ca²⁺-induced Ca²⁺ Release and Exocytosis in Pancreatic β -Cells"

Rangarajan, S.; Enserink J.M.; Kuiperij, H.B.; de Rooij, J.; Price, L.S.; Schwede, F.; Bos, J.L., *J. Cell Biol.*, **160**, 487 - 493 (2003): "Cyclic AMP Induces Integrin-mediated Cell Adhesion Through Epac and Rap1 Upon Stimulation of the β_2 -Adrenergic Receptor"

Eliasson, L.; Ma, X.S.; Renström, E.; Barg, S.; Berggren, P.O.; Galvanovskis, J.; Gromada, J.; Jing, X.J.; Lundquist, I.; Salehi, A.; Sewing, S.; Rorsman, P., *J. Gen. Physiol.*, **121**, 181 - 197 (2003): "SUR1 Regulates PKA-independent cAMP-induced Granule Priming in Mouse Pancreatic B-cells"

Bos, J.L., *Nature Rev. Mol. Cell Biol.*, **4**, 733 - 738 (2003): "Epac: A New cAMP Target and New Avenues in cAMP Research"

Christensen, A.E.; Døskeland, S.O., *Handbook of Cell Signaling*, Vol. **2**, Academic Press/Elsevier Science, San Diego, CA, 549 - 554 (2003): "Cyclic Nucleotide Analogs as Tools to Investigate Cyclic Nucleotide Signaling"

Kopperud, R.; Krakstad, C.; Selheim, F.; Døskeland, S. O., *FEBS Lett.*, **546**, 121 - 126 (2003): "cAMP Effector Mechanisms. Novel Twists for an 'Old' Signaling System"

Enserink J.M.; Christensen, A.E.; de Rooij, J.; van Triest, M.; Schwede, F.; Genieser, H.-G.; Døskeland, S.O.; Blank, J.L.; Bos, J.L., *Nature Cell Biol.*, **4**, 901 - 906 (2002): "A novel Epac-specific cAMP Analog Demonstrates Independent Regulation of Rap1 and ERK"