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Technical Information about Sp-8-Br-cAMPS-AM

Membrane-permeant, metabolically activatable stimulator of cAMP-dependent protein kinase type I & II

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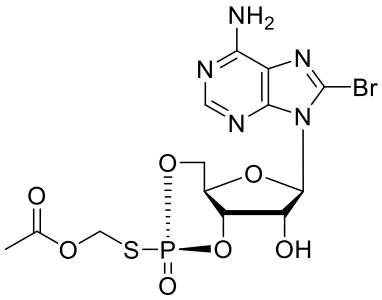

Abbreviation: **Sp-8-Br-cAMPS-AM**

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
$C_{13}H_{15}BrN_5O_7PS$	[pending]	496.2	λ_{max} 264 nm / ε 17000 / pH 7	B 029

Name: 8- Bromoadenosine- 3', 5'- cyclic monophosphorothioate, Sp-isomer, acetoxymethyl ester

Description: Sp-8-Br-cAMPS-AM is an analogue of the natural signal molecule cyclic AMP, in which the hydrogen in position 8 of the heterocyclic nucleobase is replaced by bromine and the polar cyclic phosphate is masked by an acetoxymethyl group. In addition, the axial of the two exocyclic oxygen atoms in the cyclic phosphate moiety is replaced by sulfur. The suffix "p" indicates that R/S nomenclature refers to phosphorus.

Properties: Sp-8-Br-cAMPS-AM is a prodrug of the PDE-resistant protein kinase A activator Sp-8-Br-cAMPS (Cat. No. B 002) which can be useful if Sp-8-Br-cAMPS is too polar for direct application. The acetoxymethyl group of Sp-8-Br-cAMPS-AM masks the charged polar phosphate and thus makes the molecule highly membrane-permeant. Inside the cell esterases release the much more polar Sp-8-Br-cAMPS which is thus accumulated. Also, minor amounts of 8-Br-cAMP might be released by chemical hydrolysis, especially if esterase activity is low in the biological system used.
Biolog also offers phosphate tris(acetoxymethyl)ester, PO_4-AM_3 (Cat. No. P 030), which is recommended as control reagent in Sp-8-Br-cAMPS-AM applications to test for side effects of enzymatically released acetic acid and formaldehyde, two metabolites with potential biological functions.

Specification: Lyophilized or crystallized solid. Please note that equal concentrations of Sp-8-Br-cAMPS-AM can appear very different in volume due to sensitivity of the lyophilized form to humidity. Micromolar quantities are determined by UV at λ_{max}.

Purity: Typical analysis is better than 97% (HPLC / UV / 264 nm) for the mixture of equatorial and axial isomers. The product is not sterile and has not been tested for endotoxins.

Solubility/Application: Due to its rather high lipophilicity, the solubility of Sp-8-Br-cAMPS-AM in water or buffers is limited. We suggest to use a small amount of anhydrous organic solvent such as anhydrous DMSO or DMF for dissolution at 1-100 mM, and to dilute with water or buffer down to the concentrations required. In some cases, especially at high concentrations (~1 mM), Pluronic® F-127 (Molecular Probes) can be useful to facilitate solubilization in physiological media. Please keep in mind that due to the high potency of Sp-8-Br-cAMPS-AM relatively low concentrations (0.005-0.1 mM) should be sufficient, and be sure to check for DMSO/DMF tolerance in your system. Since Sp-8-Br-cAMPS-AM is bioactivated by esterases, application to cell cultures should be performed without serum supplements (even heat-inactivated serum still contains active esterases!) in the media for at least 15 minutes. Otherwise, serum esterases may strongly reduce the cell-loading efficacy. Please rinse tube walls carefully and preferably use ultrasonic or vortex to achieve total and uniform mixing.

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Stability and Storage: Sp-8-Br-cAMPS-AM is sufficiently stable to be shipped at ambient temperature, however, it should be stored in the freezer (-20°C necessary, -80°C recommended). Please note that aqueous solutions are rather labile and should be freshly prepared immediately before use. Stock solutions in anhydrous DMSO or DMF should be relatively stable when stored frozen at -20°C to -80°C. Please do not use ethanol or other potentially reactive organic solvents for stock solutions or storage.

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 AM-modified Cyclic Nucleotides:

Wolter, S.; Kloth, C.; Golombek, M.; Dittmar, F.; Försterling, L.; Seifert, R., Biochem. Pharmacol., 98, 119 – 131 (2015): " cCMP Causes Caspase-dependent Apotosis in Mouse Lymphoma Cell Lines"

Chepurny, O.G.; Leech, C.A.; Kelley, G.G.; Dzhura, I.; Dzhura, E.; Li, X.; Rindler, M.J.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *J. Biol. Chem.*, **284**, 10728 - 10736 (2009): "Enhanced Rap1 Activation and Insulin Secretagogue Properties of an Acetoxymethyl Ester of an Epac-selective cyclic AMP Analog in Rat INS-1 Cells: Studies with 8-pCPT-2'-O-Me-cAMP-AM"

Vliem, M.J.; Ponsioen, B.; Schwede, F.; Pannekoek, W.-J.; Riedl, J.; Kooistra, M.R.H:; Jalink, K.; Genieser, H.-G.; Bos, J.L.; Rehmann, H., *ChemBioChem.*, **9**, 2052 - 2054 (2008): "8-pCPT-2'-O-Me-cAMP-AM: An Improved Epac-selective cAMP Analogue"

Krakstad, C.; Christensen, A. E.; Døskeland, S. O., *J. Leukoc. Biol*., **76**, 641 - 647 (2004), Epub 2004 Jun 04: “cAMP Protects Neutrophils Against TNF-alpha-induced Apoptosis by Activation of cAMP-dependent Protein Kinase, Independently of Exchange Protein Directly Activated by cAMP (Epac)”

Kruppa, J.; Keely, S.; Schwede, F.; Schultz, C.; Barrett, K.E.; Jastorfff, B.;, *Bioorg. Med. Chem. Lett*., **7**, 945 - 948 (1997): "Bioactivatable Derivatives of 8-substituted cAMP Analogues"

Brustugun, O.T.; Mellgren, G.; Døskeland, S.O., Proc. 10th Protein Kinase Seminar, Lillehammer, Norway 1996, A 2: "Activation of Cyclic AMP Dependent Protein Kinase in Swiss 3T3 Fibroblasts Evokes a Triphasic Response"

Schultz, C.; Vajanaphanich, M.; Genieser, H.-G.; Jastorff, B.; Barrett, K.E.; Tsien, R.Y., *Mol. Pharmacol*., **46**, 702 - 708 (1994): "Membrane-permeant Derivatives of Cyclic AMP Optimized for High Potency, Prolonged Activity, or Rapid Reversibility"

Schultz, C.; Vajanaphanich, M.; Harootunian, A.T.; Sammak, P.J.; Barrett, K.E.; Tsien, R.Y., *J. Biol. Chem*., **268**, 6316 - 6322 (1992): "Acetoxymethyl Esters of Phosphates, Enhancement of the Permeability and Potency of cAMP"

Selected References for the related PDE-resistent Sp-cAMPS-AM (Cat. No. A 035):

Thibault, N.; Burelout, C.; Harbour, D.; Borgeat, P.; Naccache, P. H.; Bourgoin, S. G., *J. Leukoc. Biol.*, **71**, 367 - 377 (2002): “Occupancy of Adenosine A2a Receptors Promotes fMLP-induced Cyclic AMP Accumulation in Human Neutrophils: Impact on Phospholipase D Activity and Recruitment of Small GTPases to Membranes“

Maronde, E.; Korf, H.-W.; Niemann, P.; Genieser, H.-G.; *J. Pineal Res*., **31**, 183 - 186 (2001): "Direct Comparison of the Potency of Three Novel cAMP Analogs to Induce CREB-phosphorylation in Rat Pinealocytes"

Genieser, H.-G.; Niemann, P.; Maronde, E., Proc. 10th Interntl. Conference Second Messenger & Phosphoproteins, Jerusalem, Israel 1998, p.124: "Membrane-permeant Precursors of Sulfur-modified, Phosphodiesterase-resistant Protein Kinase A Activators"

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