# COUMARIN-CAGED FORSKOLIN FOR TIME-CONTROLLED CELL-BASED CAMP EXAMINATIONS

Ref-Nr: TA-PT 1.2859

## HINTERGRUND

“Caged compounds” are chemically modified molecules that release e.g., biologically active substances when irradiated with light of defined wavelengths. The main field of application is in biochemical and cell biological research.[i] [ii] Biologically active compounds are protected with a photolabile group ("cage") and thereby become biologically inactive. The protective group can be photochemically removed and the previously inactive compound regains its biological activity. Caged compounds can be used to release effectors in a temporally and/or spatially restricted manner. This is advantageous when either the direct application of the compound is difficult (e.g. inside a cell) or too slow to achieve the relevant concentration. The caged compound, on the other hand, may accumulate in the cell by diffusion. Release of the effector by photolysis can then be achieved very fast. By using flash lamps or lasers it is possible to perform time-resolved analyses (pico to milliseconds) of biochemical processes, e.g., enzymatically catalyzed reactions or signal propagation. Caged compounds can be used, e.g., to investigate the time dependency of G-protein coupled receptor (GPCR)-mediated signaling cascades. G-protein-coupled receptors (GPCRs) form the largest family of membrane-bound receptors that regulate a large number of cellular and medically relevant processes. Activation of GPCRs typically causes changes in second messenger concentrations, e.g., of cyclic adenosine-3 ', 5'-monophosphate (cAMP) or calcium ($Ca^{2+}$). The naturally occurring diterpene forskolin is used as a stimulator of membrane-bound ACs in experimental biochemistry and pharmacology.[iii] [iv] Activation of the enzyme causes conversion of adenosine triphosphate (ATP) to the second messenger cAMP. In this way, forskolin acts downstream to GPCR activation.
Coumarin-caged forskolin derivatives are not yet known, but coumarin is an established cage group, e.g., of caged mRNA and DNA.[v].

[i] Ellis-Davies GCR.; Nat. Methods 2007, 4, 619–628.

[ii] Adams SR., Tsien RY.; Annu. Rev. Physiol. 1993, 55, 755–784.

[iii] Takeuchi K., Takehara K., Kato S., Yagi K.; Am. J. Physiol. 1997, 272, 646–653.

[iv] Kamenetsky M., Middelhaufe S., Bank EM., Levin LR., Buck J., Steegborn C.; J. Mol. Biol. 2006, 362, 623–639.

[v] Chaulk SG., MacMillan AM.; Nucleic Acids Res. 1998, 26, 3173–3178.

Forschungszentrum Jülich GmbH

Dennis Oliveira
+49 2461/61-96831
d.oliveira@fz-juelich.de
www.fz-juelich.de

## ENTWICKLUNGSSTAND

Marktreife

## PATENTSITUATION

PCT anhängig

## CATEGORIES

//Chemie //Molekularbiologie, Genetik //Life Sciences //Neue Substanzen

## PROBLEMSTELLUNG

Forskolin is used experimentally as an activator of membrane-bound adenylyl cyclases (ACs). However, in order to activate the enzyme, forskolin has to passively cross the membrane. Since this process takes tens of seconds, the activation kinetics are rather smooth. So far, no water-soluble, caged forskolin derivatives were available that specifically and quickly release the biologically active compound upon photolysis.

## LÖSUNG

Coumarin-caged forskolin is synthesized in a 15-step protocol from protected forskolin and a coumarin derivative functionalized with N-methylethylenediamine (Fig. 1A, scheme 1). Coumarin-caged forskolin can be cleaved by irradiation with light to the biologically active product (Fig. 1A, scheme 2 and Fig. 1B) and thus qualifies for the described biological application.

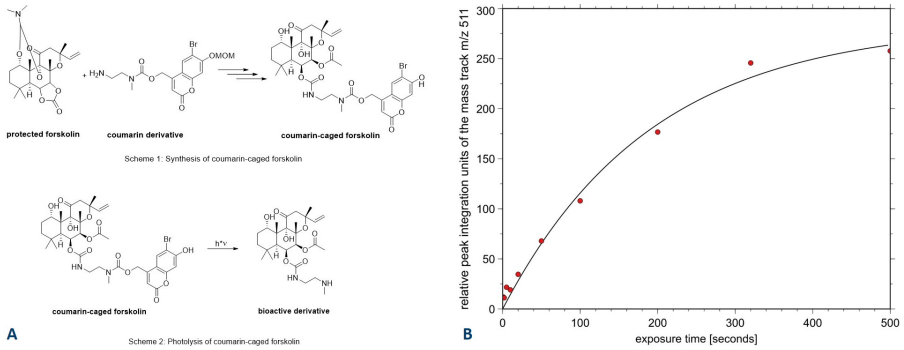


Fig.1: A) Sythesis and photolysis of coumarin-caged forskolin. B) Photolysis of coumarin-caged forskolin to the bioactive derivative (m/z = 511).

## VORTEILE

It is possible for the first time to carry out temporally controlled cAMP measurements with a forskolin derivative in the interior of living cells.

## ANWENDUNGSBEREICHE

Detailed temporal investigation of AC-mediated signaling.