Lipophilically, Bio- or Photocleavable Masked Cyclic Nucleoside Monophosphate Derivatives - Tools for Non-invasive Cell Assays

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Background

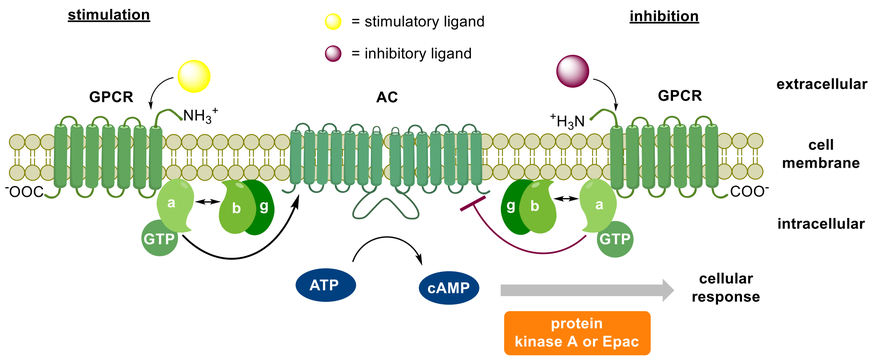


Figure 1: cAMP biosynthesis and regulation of its levels.

One of the key factors in cell signaling is the second messenger cyclic adenosine 3',5'-monophosphate (cAMP). Its biosynthesis and signal transduction pathways are shown in Figure 1. However, the role and mechanism of action of different cNMPs in T-cell regulation are not yet fully elucidated. A better understanding could enable the identification of novel targets for the treatment of autoimmune diseases. In cell studies with cAMP or other cNMPs, it is difficult to deliver these molecules, as their high polarity excludes passive membrane transport. Invasive methods such as patch clamp, electroporation, or microinjection are unsuitable because they can cause stress reactions. Therefore, membrane-permeable pronucleotide systems have been developed (Figure 2). The most common protecting groups are acetoxymethyl (AM) or benzyl esters, but they are chemically unstable and only suitable for certain cell types and nucleosides.[1,2]

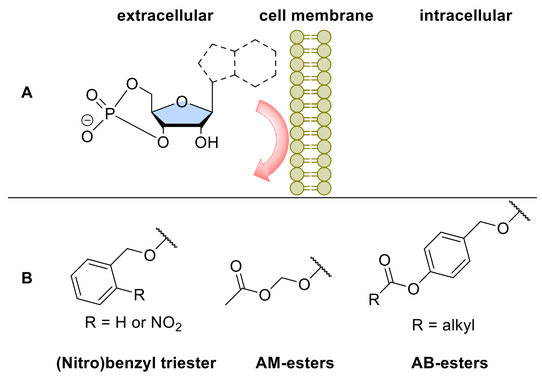


Figure 2: A) cAMP is unable to penetrate the cell membrane directly. **B)** Pronucleotide masking technologies are employed to facilitate cellular uptake.

A New Generation of Masking Units

Biocleavable-PG

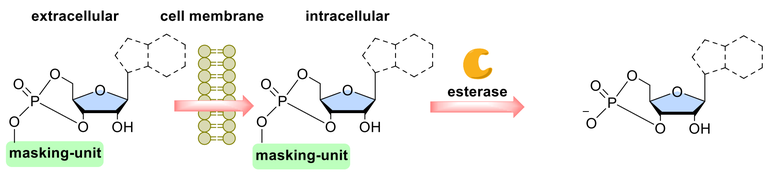


Figure 3: Biocleavable pronucleotide concept as strategy to enable a passive membrane transport of phosphorylated nucleotides.

Over the past 20 years, the Meier research group has successfully adapted the acyloxybenzyl (AB) prodrug system for a variety of nucleosidic target molecules. Hydrolysis experiments demonstrated that these compounds exhibit high chemical stability, along with rapid and selective enzymatic cleavage. To modulate the ability and rate of the pronucleotide crossing membranes, the chain length of the aliphatic acyl residue was altered. With increasing chain length, both the permeability and stability of the prodrug improved.[3]

Photocleavable-PG

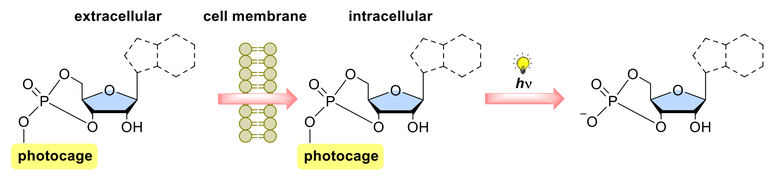


Figure 4: Photolabile protecting groups to mask negative charges of phosphorylated nucleotides.

Photocages are a class of prodrugs containing photolabile protecting groups (PPGs) that can be cleaved by irradiation with a specific wavelength of light. The cleavage is highly selective, controllable, and bioorthogonal, enabling spatiotemporal release of the compound. By varying the intensity and wavelength of the light, the release process can be easily modulated. The photocages that have been most extensively studied in chemistry and biochemistry are based on o-nitrobenzyl and coumarin PPGs.[4]

Bio- & photocleavable-PG

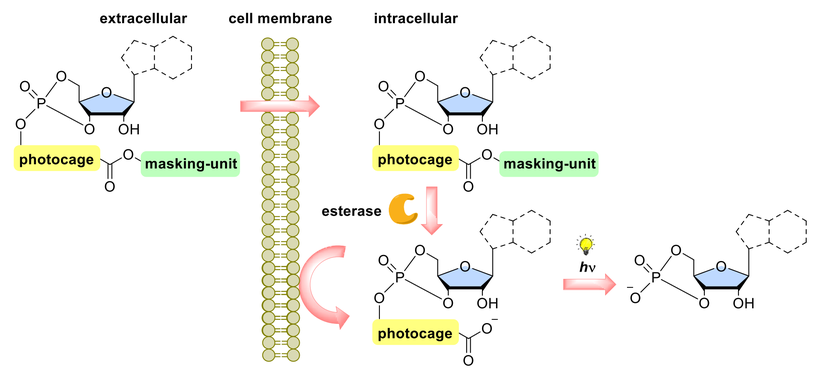
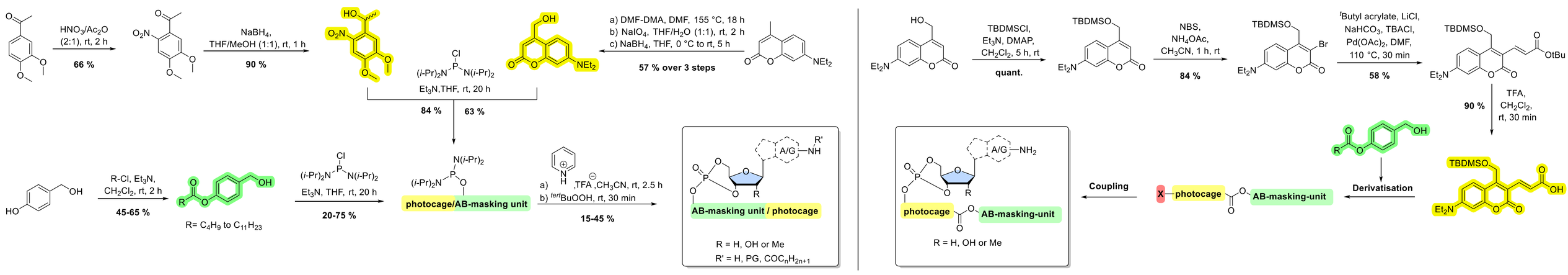


Figure 5: New, bi-functional masking units, a combination of biocleavable and photocleavable masking units.

To enhance spatiotemporal control and achieve a lock-in effect, we combined a biocleavable masking unit with a photocleavable unit (a bi-functional masking unit). Enzymatic cleavage enables the accumulation of the negatively charged PPG in specific cellular compartments.[5]

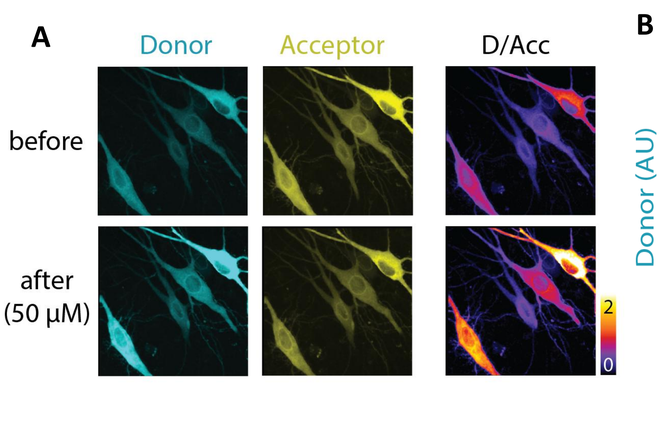
General Synthesis of Membrane Permeable cNMP Derivatives



Conclusion & Future Perspectives

We introduced different masking units via a short and flexible chemical route, resulting in a small compound library of AB-masked cNMPs. Hydrolysis tests demonstrated that the AB-cNMPs possess high chemical stability, while rapid cleavage was observed following esterase activation. A FRET-based (Förster resonance energy transfer) cell assay in cardiomyocytes and hippocampal neurons confirmed rapid cellular uptake of AB-cNMPs and esterase-catalyzed activation, leading to an almost immediate cellular response.

To evaluate our photocaged cNMP derivatives (methyl-nitroveratryl photocages), a FRAP (Fluorescence Recovery After Photobleaching) system was used to monitor Ca^{2+} release within cells. The experiment demonstrated a rapid cellular response following stimulation with a 405 nm flash of light, which cleaved the photoactive bond and released cAMP. This indicates successful passive permeation of the compound into the cell and subsequent release.



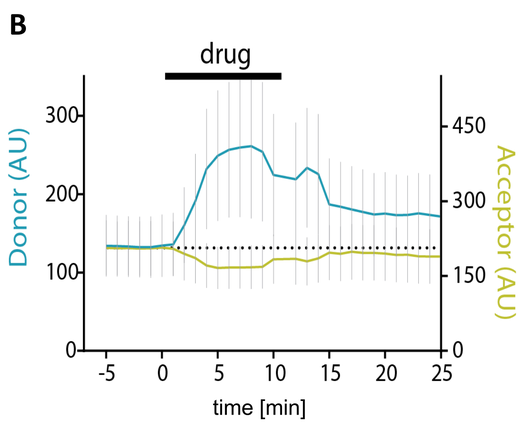
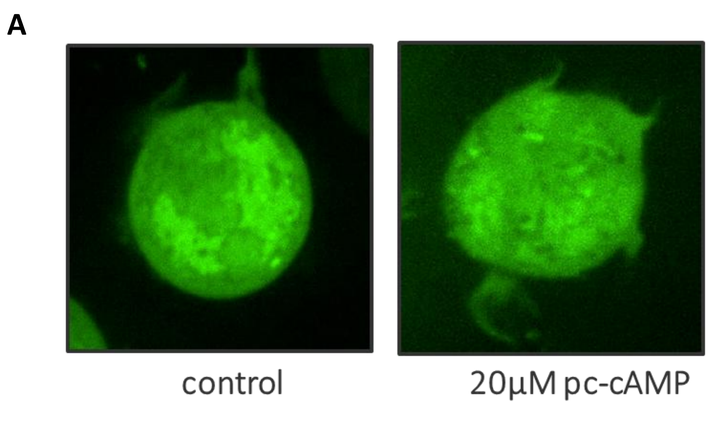


Figure 6: A) Hippocampal tissue before and after stimulation with cAMP. **B)** Donor/acceptor channel emission and normalized FRET ratio during the addition of OB-N(Bu)-2'O(Me)-cAMP to hippocampal neurons transfected with the FRET sensor H188. The increase in the FRET ratio following the addition indicates a cellular release of cAMP and its binding to the FRET biosensor.



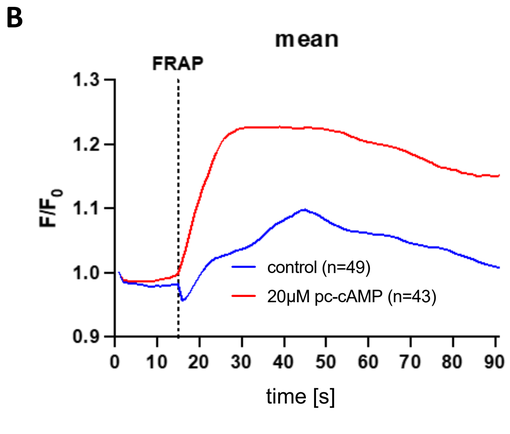


Figure 7: A) Cells loaded with the Ca^{2+}-sensitive dye Fluo-4 after stimulation at 405 nm. FRAP measurements were performed at 488 nm. **B**) Fluorescence ratio used to compare control and photocaged cAMP (pc-cAMP).

Driven by the positive results and the goal of achieving a bathochromic shift—thus using wavelengths less harmful to cells—initial synthesis steps towards thio-coumarin photocages were undertaken. To better understand the structure-activity relationship and to enhance membrane permeability, the library of synthesized cNMP compounds is continuously being expanded with variations of lipophilic side chains.

Collaboration & Funding

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References

[1] C. H. Serezani, M. H. Ballinger, D. M. Aronoff, M. Peters-Golden, „Cyclic AMP: Master regulator of innate immune cell function" *Am. J. Respir. Cell. Mol. Biol.*, **2008**, *39*, 127-132.
2 A. Ruthenbeck, E. Marangoni, B. P. Diercks, A. Krüger, A. Froese, N. I. Bork, V. O. Nikolaev, A. H. Guse, C. Meier "Membrane-permeable octanoyloxybenzyl-masked cNMPs as novel tools for non-invasive cell assays" *Molecules*, **2018**, *23*(*11*), 2960.
[3] T. Gollnest, T. Dinis de Oliveria, A. Rath, I. Hauber, D. Schols, J. Balzarini, C. Meier "Lipophilic prodrugs of nucleoside triphosphates as biochemical probes and potential antivirals" *Nat. Commun.*, **2015**, *6*, 8716.
[4] P. Klán, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz "Photoremoval protecting groups in chemistry and biology: Reaction mechanisms and efficacy" *Chem. Rev.* **2013**, *113*, 119-191.
[5] M. R. Clotworthy, J. J. M. Dawson, M. D. Johnstone, C. L. Fleming "Coumarin-derived caging groups in the spotlight: Tailoring physiochemical and photophysical properties" *ChemPlusChem*, **2024**, *89*, e202400377.