Total synthesis of lipophilic masked NAADP derivatives

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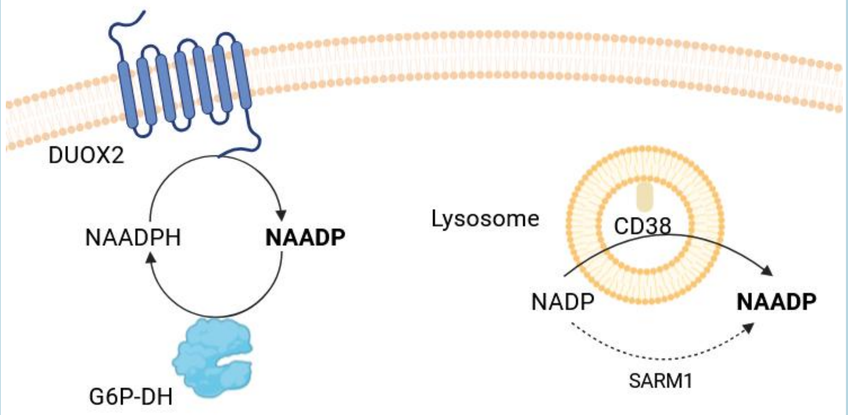
Abstract

Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent Ca^{2+}-releasing second messenger.[1] The release of Ca^{2+} by NAADP was verified in different eukaryotic cell types, for example in human T-lymphocytes.[2] To study its cellular effects, NAADP must be present in the cells, which is challenging due to its polarity. In our group, a new **masked compound** was developed: **Membrane permeAble STabilized bio-rEversible pRotected NAADP** (MASTER-NAADP)[3]
The MASTER concept introduced several modifications leading to a **higher stability** compared to previously applied masking concept, e.g. the AM-approach.[3]

Biosynthesis pathways of NAADP[4]

Proposed biosynthesis pathways of NAADP e.g. after binding of an antigen presenting cell to a T-cell



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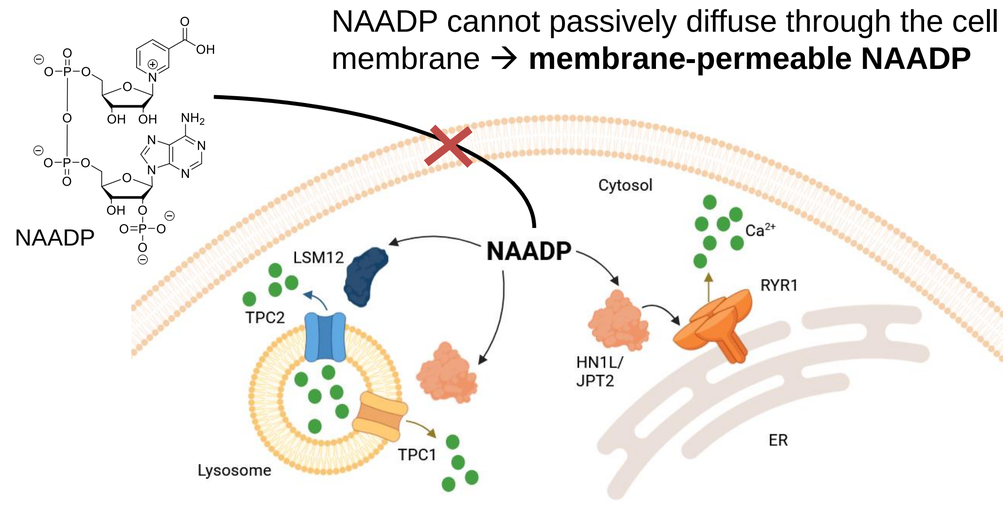
DUOX2: dual oxidase 2
NAADP: Nicotinic acid adenine dinucleotide phosphate
NADP: Nicotinamide adenine dinucleotide phosphate
G6P-DH: glucose 6-phosphate dehydrogenase

NAADP induced Ca^{2+}-release[4]

NAADP cannot passively diffuse through the cell membrane → **membrane-permeable NAADP**



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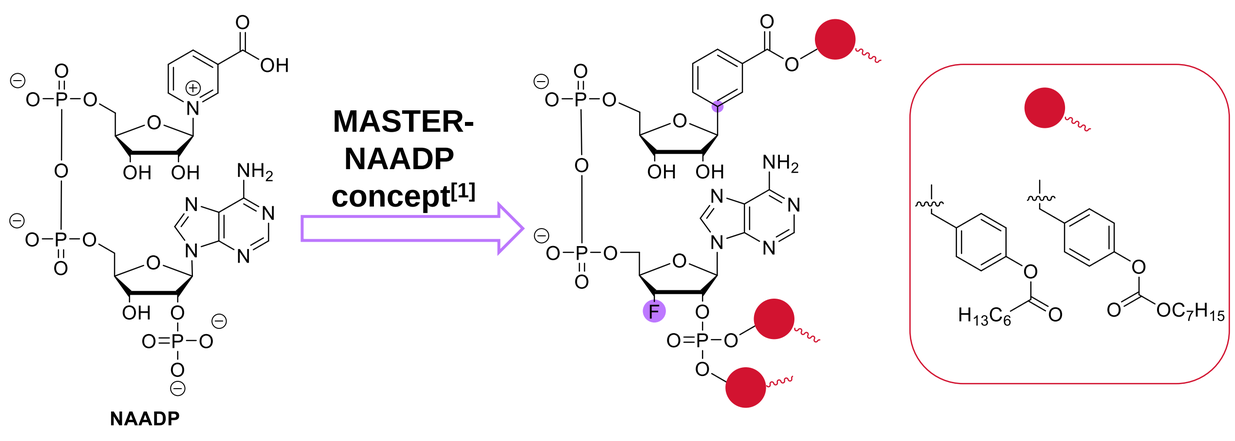
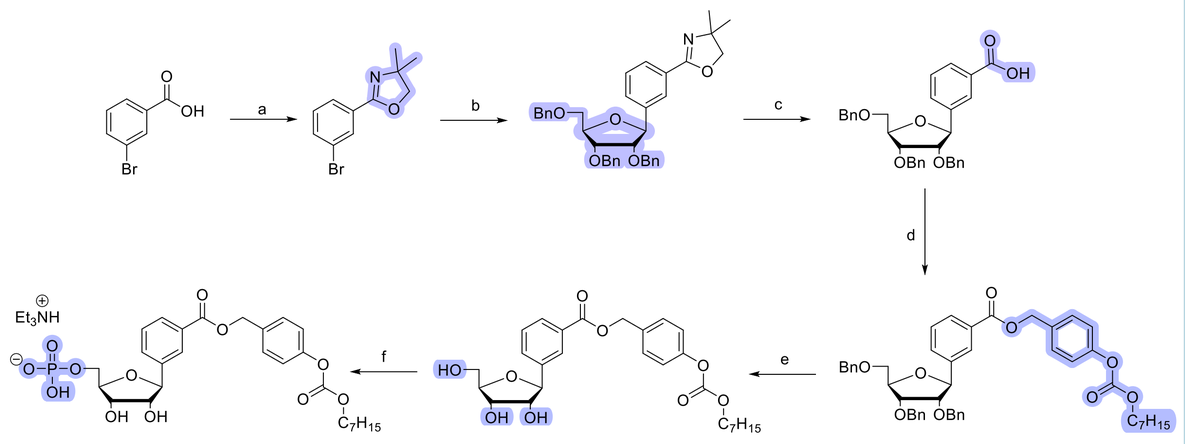
LSM12: Sm-like 12
TPC2: two-pore channel 2
HN1L/JPT2: 1-like/Jupiter microtubule-associated homolog 2
RYR1: Ryanodine receptor 1

Objective

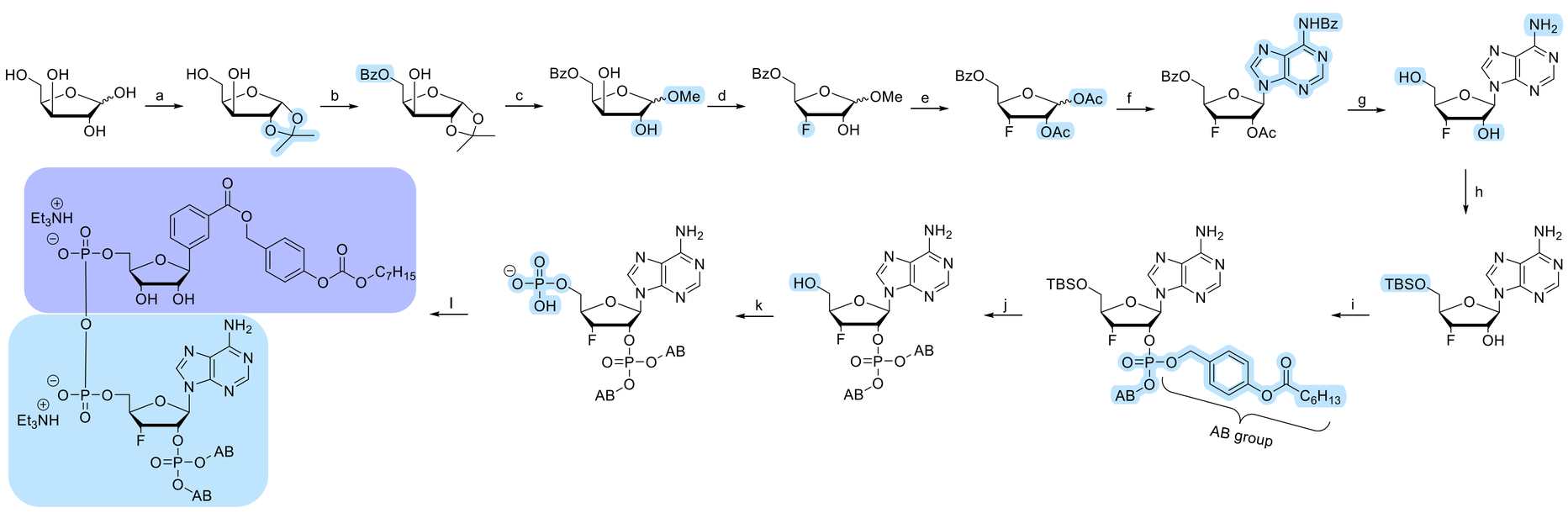


Synthesis "Northern" building block[3,5]



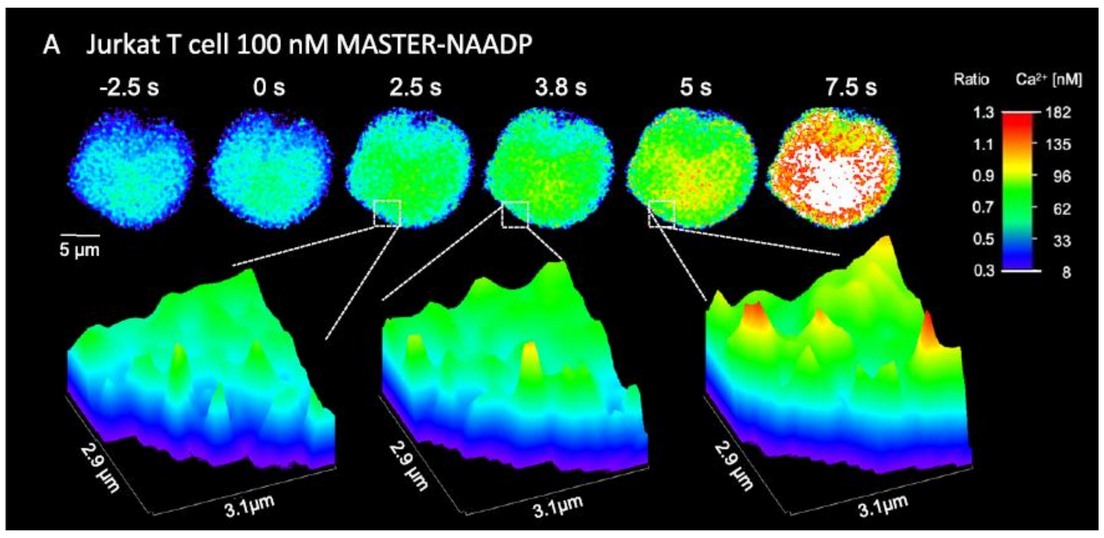
a) (i) $SOCl_2$, cat. DMF, 80 °C, 2 h, (ii) 2.0 eq. 2-amino-2-methyl propanol, CH_2Cl_2, rt, 2 h, (iii) $SOCl_2$, rt, 16 h, 83 %; b) (i) 2.3 eq. ^{t}BuLi, THF, - 78 °C, 45 min, (ii) 1.2 eq. $LaCl_3{\cdot}2LiCl$, THF, - 78 °C, 0.5 h, (iii) 1.0 eq. 2,3,5-tri-O-benzyl-ribono-γ-lactone, THF, - 78 °C - rt, 18 h, (iv) 3.0 eq. Et_3SiH, 2.5 eq. $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C - rt, 18 h, 49 %; c) (i) CH_3NO_2/MeI 2:1, reflux, 20 h, (ii) MeOH/KOH aq. (20 %) 1:1 v/v, reflux, 20 h, quant.; d) (i) 1.5 eq. TCBC, 1.5 eq. Et_3N, THF, rt, 0.5 h, (ii) 1.2 eq. ACB-mask, 0.30 eq. DMAP, THF, 70 °C, 3 h, rt, 16 h, 47 %; e) 3.5 eq. BCl_3 (1 M in CH_2Cl_2), CH_2Cl_2, - 78 °C - 0 °C, 2 h, 73 %; f) (i) 7.5 eq. $POCl_3$, 2.0 eq. proton sponge, TMP, 0 °C, 3.5 h, (ii) 26 eq. TEAB (1 M), (iii) RP-column, (iv) ion-exchange, (v) RP-column, 46 %.

Synthesis "Southern" building block and coupling of the NAADP derivative[3,5]

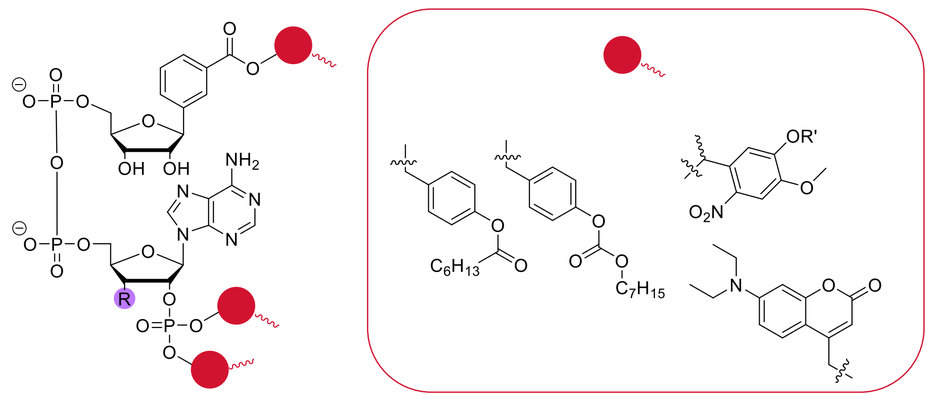


a) (i) 0.10 eq. $CuSO_4$, 2.8 eq. conc. H_2SO_4, acetone, 3h, rt, (ii) HCl-solution (0.2%), 6h, rt, 99%; b) 1.5 eq. Et_3N, 1.1 eq. BzCl, CH_2Cl_2, 1 h, 0 °C, 92 %; c) 0.2 eq. I_2, MeOH, 4 h, 70 °C, 86 %; d) 1.5 eq. DAST, dry CH_2Cl_2, 20h, - 78°C - rt, 43 %; e) 4.7 eq. Ac_2O, 4.7 eq. conc. H_2SO_4, AcOH, 19 h, rt, 93 %; f) (i) 1.5 eq. *N*-benzoyladenine, 1.2 eq. BSA, $C_2H_4Cl_2$, 1 h, 90 °C, (ii) 1.0 eq. ribose derivative, 4.0 eq. TMSOTf, $C_2H_4Cl_2$, 5.5 h, 90 °C, 61 %; g) ammonia 7 N in MeOH, 28 h, 70 °C, 84%; h) 1.5 eq. TBSCl, dry pyridine, 24 h, rt, 71 %; i) (i) 1.3 eq. amidite, 1.2 eq. pyridinium trifluoracetate, CH_2Cl_2, 50 min, rt, (ii) 1.5 eq. tert-butylhydroxyperoxide (5.0M in decane), 0 °C, 1 h, 87 %; j) 5.2 eq. triethylamine-trihydro-fluoride, CH_2Cl_2, 19h, rt, 81 %; k) 4.0 eq. $POCl_3$, TMP, 6.5 h, 0 °C, 31 %; l) (i) 16 eq. Et_3N, 20 eq. TFAA, CH_3CN, 0 °C, 15 min, (ii) 1.0 eq. **"northern" monophosphate**, CH_3CN, 0 °C, 15 min, (iii) 6 eq. NMI, 10 eq. Et_3N, CH_3CN, rt, 15 min, (iv) 0.9 eq. **diphosphate**, CH_3CN/DMF 5:1, rt, 3 h, 57 %.

Ca^{2+}-release in Jurkat T cells[3]



Outlook



References

[1] R. Aarhus, R. M. Graeff, D. M. Dickey, T. F. Walseth, C. L. Hon, *Journal of Biological Chemistry* **1995**, *270*, 30327; [2] A. Gasser, S. Bruhn, A. H. Guse, *Journal of Biological Chemistry* **2006**, *281*, 16906; [3] S. Krukenberg, F. Möckl, M. Weiß, P. Dekiert, M. Hofmann, F. Gerlach, K. J. Winterberg, D. Kovacevic, I. Khansahib, B. Troost et al., *Nat Commun* **2024**, *15*, 8008; [4] A. H. Guse, *Cells* **2022**, *11*; [5] M. Yoshikawa, T. Kato, T. Takenishi, *Tetrahedron Letters* **1967**, *8*, 5065.